Attacks on Muscle

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UP TO THE PRESENT CENTURY muscle was the favorite object of physiological research. An enormous amount of work carried on by classical methods was repeated later with perfected physical methods. The results of such refinements failed to leave in their wake a deeper understanding than had been obtained before. The reason lies in the wholistic nature of approach. To the physiologist muscle and a muscle twitch are units which cannot be disintegrated.

Attempts to understand muscle by breaking it down to its molecular components date back to the sixties, when W. Kühne (*7*) prepared from muscle a globulin-like protein, myosin, supposedly derived from contractile fibril. Leading contemporaries, Danilevski, (*2*) Halliburton (*5*), and v. Fürth (*11*), corroborated and extended the observation. In the thirties of our century the study of myosin was again taken up by more modern methods (*12*).

Though interesting and specific relations of myosin and ATP were discovered (*4*), the mechanism of contraction and the role of myosin remained obscure.

A third line of attack was taken up in the author's laboratory ten years ago at Szeged and later at Budapest, Hungary. This line was based on the belief that such a complex mechanism as the contracting muscle fibril can be understood neither by keeping it as a whole nor by decomposing it to molecules. It was believed that an understanding could be achieved by breaking the system down gradually, dismantling it step by step, always trying to put together again what we have taken to pieces. It was further believed that no single method of investigation could give the desired information. All methods available had to be employed in one single coordinated effort.

Two years ago the author started organizing such an attempt in this country. Part of this research group is working at the National Institutes of Health at Bethesda, Maryland, and part at the laboratory of the Institute of Muscle Research at the Marine Biological Laboratory in Woods Hole, Massachusetts. In the middle of August the two groups convened at Woods Hole to discuss their problems and methods, the meeting being attended by a few guests interested in muscle. Out of these discussions a new conception of muscular contraction seems to have emerged.

The contractile structure is built of a protein complex, "actomyosin," discovered at Szeged. The "myosin" partner of this complex is in many ways different from earlier myosin and was crystallized by the author. Actin was isolated by F. B. Straub (*12*). In itself, neither actin nor myosin shows any sign of contractility, nor does actomyosin in itself. What makes it work is the master substance of muscle, ATP.

Adenosine triphosphate is a water-soluble nucleotide consisting of small molecules. Its existence was only gradually recognized. Embden (*3*) described adenosine phosphate and Meyerhof and Lohmann (*8, 9*) showed that in muscle adenosine was linked not to one but to three phosphate groups; hence the name ATP, abbreviation for adenosine triphosphate. This ATP contains, in the links holding its phosphate groups together, 11,000 cals of free energy which, according to our present knowledge, is the sole immediate source of the energy of muscular contraction. But ATP is not merely a store of energy. It dominates the whole physical state of muscle. Without it muscle is not only inactive but is also stiff and inelastic. Muscle in *rigor mortis* is actually muscle with no ATP.

Myosin, if not linked to actin, has one striking property, its great affinity to ions. It binds ions very strongly so that its physical state, charge, and solubility depend on the quality and quantity of ions present. Though it is a hydrophylous colloid, myosin is discharged and quantitatively precipitated by 0.025 M KCl or NaCl. It has an especially great affinity to Mg, which is always present in muscle in high concentration. Myosin, by binding Mg and K, develops a high affinity to ATP, with which it links up to a fairly stable complex, "myosin-ATP."

Actin, too, has its striking peculiarities. The most surprising one is that it can exist in two forms, in the form of globules and in the form of long threads.

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If extracted by Straub's method, it is in the globular form (G-actin) consisting of small, round molecules of mol. wt 70,000 g. If a small quantity of salt, KCl or NaCl, is added in the presence of traces of Mg, the globules unite to long threads, studied first under the electron microscope by Jakus and Hall (*6*). These relatively thick threads give no information about their finer structure, or the mechanism of the building of threads out of globules. If, however, thread formation is induced under special conditions, as it was by G. Rozsa, the author, and R. W. G. Wyckoff (*10*) at the National Institutes of Health, actin forms very thin molecular threads. If these threads come to lie side by side they develop a cross striation. The distances of this striation can be exactly measured, allowing us to draw some conclusions as to what has happened. What we find is that the small actin globules of mol. wt 70,000 g have united to form bigger units, about twenty of them now forming one particle of mol. wt about 1,500,000 g. These units are slightly elongated, 100 A wide and 300 A long; they associate end-to-end to form long fibers. If several fibers come to lie close to one another the particles of the neighboring threads associate also with their broad side.

Myosin consists of very thin and long molecules. If these are added to fibrous actin the two colloids unite to form a complex. The slender, long myosin filaments attach themselves to the actin filament lengthwise. The reactions of this complex are rather sluggish. If ATP is added it links up with the myosin to form an actomyosin-ATP complex which has the most amazing reactivity. The reactions depend now on the salt concentration. At a high salt concentration the complex dissociates into its components, actin and myosin-ATP. By changing the salt concentration by no more than 0.01 M we can make actin and myosin-ATP unite or dissociate at will. The ionic concentrations of intact muscle are such that the system is balanced on a razor edge and the two proteins just do not unite. The shift in ionic concentration, necessary for their union, is brought about by the wave of excitation and the concomitant electric current.

In the absence of ATP the actomyosin filaments are stable in their straight, stretched condition. Actomyosin-ATP is unstable in this condition. When actin and myosin-ATP unite they lose their charge and therewith they dehydrate and tend to shrink and fold up, going over into a shorter, more stable, and energy-poorer state.

If we do not allow the system to shorten by holding its ends fixed, tension will be developed. Using thermodynamic language we could also say that, by uniting, the two proteins acquire a thermodynamic potential which is dissipated in shortening. Using the language of colloidal chemistry we can also say that contraction is a mutual precipitation of two proteins, or a precipitation of a protein complex by ions, since ions are also involved.

The reaction in which the potential is developed is thermodynamically reversible; it is thus an equilibrium reaction. From the temperature dependence of its constant, free energy changes can be calculated which are in agreement with experiments measuring the work actually done. In these reactions the actin, together with the corresponding quantity of myosin, forms independent units, which react in an all-or-none fashion, that is, either react completely or not at all. Muscular activity is the sum of the independent function of these little units, the size and energy changes of which can be calculated with fair accuracy.

While contraction seems to be clearing up, we still know very little about relaxation and energy coupling. We do not know how the muscle goes back to its resting state and at which point of the cycle the ATP is split and how its energy is utilized by the protein. A recent discovery of F. B. Straub, corroborated and extended by W. Bowen and K. Laki (*1*), together with the author's earlier observations, offers the possibility of a tentative explanation. Straub found that ATP is involved in the polymerization of actin. The author observed that fibrous actin is broken up into globules during contraction of actomyosin, that actin in its globular form dissociates from myosin-ATP much more readily than fibrous actin, and that myosin greatly catalyzes the rearrangement of actin globules to fibers. Hitching all this together the following picture can be made: ATP is instrumental in holding the actin-globules together in fibers. This ATP is split in contraction by the myosin (which is known to have ATP-ase activity). The actin fiber thus falls into globules which dissociate from the the myosin-ATP. The actin globules unite spontaneously to fibers again while myosin-ATP molecules hydrate and stretch out, since myosin-ATP, if not attached to actin, hydrates spontaneously at the ionic concentration of muscle.

In this picture the difficult problem of energy coupling ("energetization") drops out altogether. If the actomyosin-ATP system contracts and performs, say, *X* calories' worth of work, we cannot expect to be able to dissociate it without using up *X* calories' worth of energy in bringing about this dissociation. (If the system dissociates it can go back spontaneously into the relaxed resting state and if we could make it dissociate without using up energy then we

would have a net profit of X calories, that is, we would have a *perpetuum mobile*.) There being no *perpetuum mobile* possible, we can state that if the muscle has done X calories of external work in contraction we need at least X calories' worth of energy to make it dissociate and relax. Since the splitting of the phosphate link of the ATP molecules holding the actin globules together entails dissociation and relaxation, we need energy to bring about its splitting. The problem is solved by nature—by enclosing into the phosphate bond the energy necessary for its later splitting. In this way the link can be split at the right moment in contraction without external help, the energy needed for this splitting having been enclosed in the phosphate bond at its formation. The final source of this energy of the "high energy phosphate bond," which has to pay for all the energy expenditure of contraction, is our food, its oxidation and fermentation.

The reader will have noticed that in developing this concept of contraction the most varied methods were used. A deeper understanding of muscular activity can be arrived at only by coordinated attack on all available lines, be they histology, electron microscopy, preparative chemistry, enzymology, colloidal chemistry, physiology, or even quantum mechanics. The meeting held at Woods Hole represents such an attack.

A deeper understanding of muscle is urgently needed for two reasons. Cardiovascular diseases, which take such a horrid toll, are mostly diseases of muscle, be it the heart muscle itself or muscle cells of the artery wall. It should not be forgotten that the immediate cause of most human death is failure of muscle, and its dysfunctioning causes a great deal of suffering. No rational therapy or prevention can be hoped for without deeper understanding of muscle. Also, we can replace missing, or worn out, parts of a mechanism only if we know about them, and so the analysis of muscle may bring to light substances of therapeutic value. The author has worked out methods for the large scale preparation of ATP making this master substance of the muscle available for medical use, and suggested its clinical trial in all conditions in which dysfunctioning of muscle is involved. Most encouraging results have been reported in various lines.

The fact that ATP is involved in the construction of the bigger functional units of actin indicates that this substance is, so to speak, a mortar of the living edifice. Thus it is imperative to try its clinical application also in degenerative diseases, like arthritis, where the basic biomolecular architecture seems to be impaired.

Relieving human suffering on a big scale may be very gratifying, but all the same, the mainspring of basic research is curiosity, the thirst for new truth and knowledge. Success in therapy is but a natural consequence of such deeper knowledge. Muscle, as a material, holds out the highest promise for those who want to understand the basic principles of life and its architecture.

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The Cortical Correlate of Pattern Vision[1]

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BOTH PHYSIOLOGISTS AND PSYCHOLOGISTS are convinced that psychological facts are directly related to certain brain processes, which determine the various characteristics of these facts. Such processes are called the cortical correlates of the phenomena in question. No actual demonstration of cortical correlates has so far been possible, and their nature has remained a matter of speculation. In the case of vision, it is true, operations of the cortex are not entirely unknown. When the organism is in a relaxed condition, the visual area of the brain, but not this area alone, exhibits the alpha rhythm, an electric oscillation of about 10 cycles per second. Since this rhythm is most pronounced when the eyes are closed, and is disturbed by visual facts, nobody regards it as the correlate of these facts as such. A few electric waves are also observed when the visual cortex is thrown into action by stimulation of the eyes; and some waves may again occur when stimulation is discontinued. But such on- and off-effects cannot be related to vision in general, because they accompany only its beginning and its end.

Many believe that cortical function is essentially of the same type as peripheral function; in other words, that nerve impulses which arrive in the cortex merely give rise to further impulses which travel in cortical fibers. This thesis meets with a serious difficulty: large masses of cortical cells tend to operate as though each were taking account of what the others are doing. Thus, Gerard (*2*) suggested that, quite apart from impulses which are transmitted in fibers, brain action may involve fields which spread through the tissue as a continuum. Later he and Libet showed that parts of the brain remain functionally interrelated even when all connecting fibers have been severed, or when their synapses are blocked by drug action (*3, 4, 8*). In the meantime, psychological evidence had led to the assumption that, when the level of activity in adjacent parts of the visual cortex differs, a direct current flows around the contour at which they are in contact (*5, 6*). An investigation of so-called figural after-effects in vision strongly supported this assumption (*7*). Just like Gerard's fields, the currents in question were supposed to spread through the brain as a volume conductor. This is not a bold assumption. The short-lived current of a nerve impulse flows through the surrounding medium no less than through the active stretch of the fiber, although in general discussions about the operations of the nervous system the fact is sometimes ignored. Again, since the alpha rhythm of human subjects is commonly registered when the electrodes are attached to the surface of the head, this activity of brain cells must also have fields which spread in the tissue as a continuum. Electric displacements which readily penetrate skull and scalp are not, of course, restricted to special conductors in the brain.

In the theory of visual perception, we cannot be satisfied with the general hypothesis that, when a subject sees objects or patterns, corresponding parts of his visual cortex are pervaded by direct currents. If it is assumed that such currents determine certain characteristics of the visual field, it will be necessary to show more specifically how these characteristics can be derived from the behavior of the cortical flow. But this is by no means an easy task. We therefore decided to postpone further theorizing until we had better evidence to support the main hypothesis, and tried to find such evidence in direct physiological tests.

Although later it will be necessary to take records from the brains of animals whose skulls have been opened, recording from the intact heads of human subjects appeared to us more convenient in a first exploration. This procedure had to give results if the size of the postulated potentials could be assumed to be comparable to the amplitude of the alpha rhythm. As our main instrument, we used the breaker type d-c amplifier constructed by the General Motors Company (*10*); the amplified current was registered with a photoelectric recorder of the General Electric Company. The subject was seated in a shielding cage. We used electrodes of the silver-silver chloride type. Usually, one electrode was placed slightly above the occipital protuberance, where the foveal region of the visual cortex is located, and the second electrode was attached to the vertex. Under these conditions, the vertex electrode was connected with the grounded parts of the amplifier and the shielding cage. Steady potentials appearing in the absence of visual stimulation were eliminated by a balancing circuit.

All currents which spread in the nervous system tend to reduce their own intensity by immediate polar-

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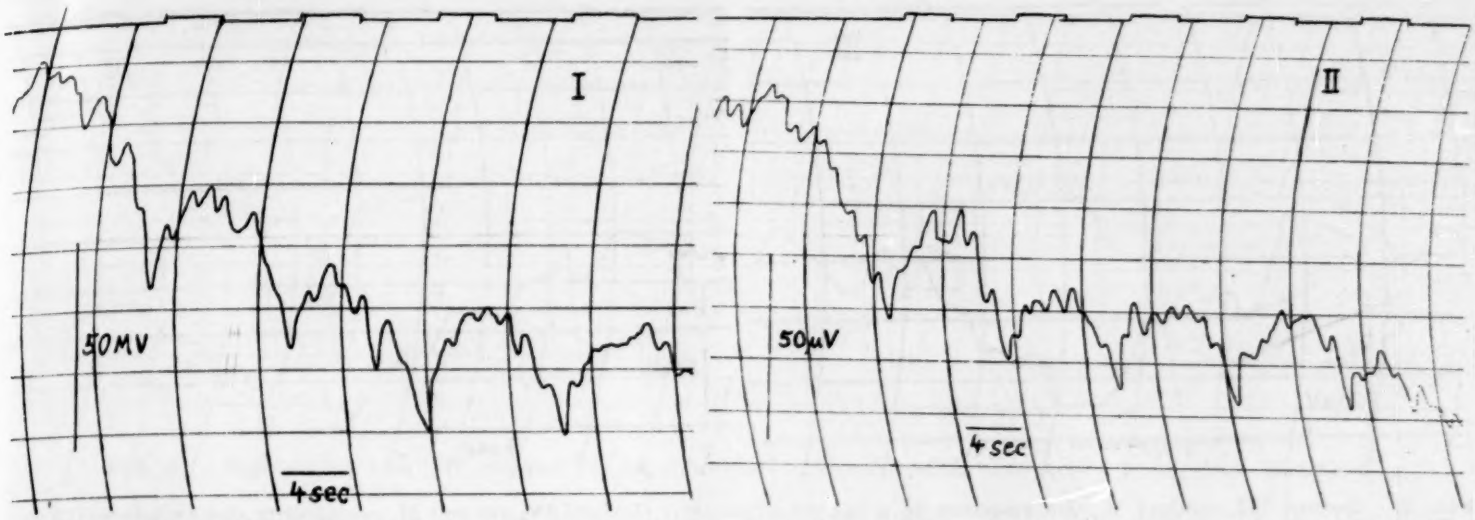

FIG. 1. Record I, subject S, four responses to four exposures of a bright moving object. Record II, subject S, five responses to five exposures of a moving object.

ization of the tissue.[1] We tried to minimize this difficulty by showing the subject bright objects that were slowly moving rather than stationary. Actually, it is more difficult, although not impossible, to obtain responses a'so in the latter situation. The objects did not move with a very high speed; we have sometimes used velocities of only 3° of visual angle per second. The records we will show here were taken with retinal speeds that varied from about 6° to 12°. It has been reported that moderately bright objects give better on-effects than very intense objects. It is our experience that objects which do not differ too strongly from their background are most likely to give good results. The reason is probably that the advantage which arises from working with a moving object is lost if the object, or rather its current, is strong enough to polarize parts of the visual area before the stimulus actually reaches these parts. We have used moving strips of white or gray cardboard, seen against a dark background in a fairly well illuminated room, as well as moving bars of light projected on a screen under conditions of lower general illumination. Of the records to be shown in this report, only the first, the second, and the fifth have been taken in the latter situation. The objects always had a vertical orientation, and moved in the horizontal direction.

When studying the on-effects of the rabbit's visual cortex, Bishop once remarked that "the variety in the records taken under as nearly constant conditions as we are able to maintain would be discouraging in experiments on any other structure than the cerebral cortex . . ." (*1*). This statement also applies to our records, which often show irregularities of unknown origin. But we should have to regard these records with suspicion if there were no such irregularities. Even in a very calm subject, processes are bound to occur which are not related, or related only indirectly, to the fact that an object moves in his visual field. We have taken records from 13 subjects altogether. All have given responses, although results have been more satisfactory with some than with others. The curves we will now discuss show responses of four different individuals.

When Record I (Fig. 1) was taken, the object moved four times in succession across a screen, in the middle of which a faint fixation mark was given. Both electrodes were placed in the median plane of the head, one in the region of the visual cortex, the other near the vertex. In examining this record, one has to keep in mind that it is written on very slowly moving paper (cf. the length of 4 sec given below the record). It will be seen that there are four responses corresponding to the four marks on top, which indicate the time and the length of the exposures. The responses are maximal in the middle of the exposures, i.e., when the object passes the fixation mark and, in the visual cortex, the place of the occipital electrode. The total length of the four exposures with the intervals between them is about half a minute. During this period the base line has drifted considerably. It is nevertheless obvious that all responses are deflections downward. With the connections of our arrangement, this means that, when the object moves across the fovea, the surface of the cortex under the occipital electrode is positive in relation to the vertex. The size of the responses amounts to slightly more than 25 μv. The small waves which the curves exhibit represent the subject's very slow and steady pulse. It appears in almost all his records, but is disturbing only with some positions of the electrodes.

Record II is a repetition of the same experiment, with the same subject, but with five successive exposures. Although in this case the base line has begun to drift before the exposures, the five responses are

[1] To be exact, not only is the tissue polarized; its polarizability also grows.

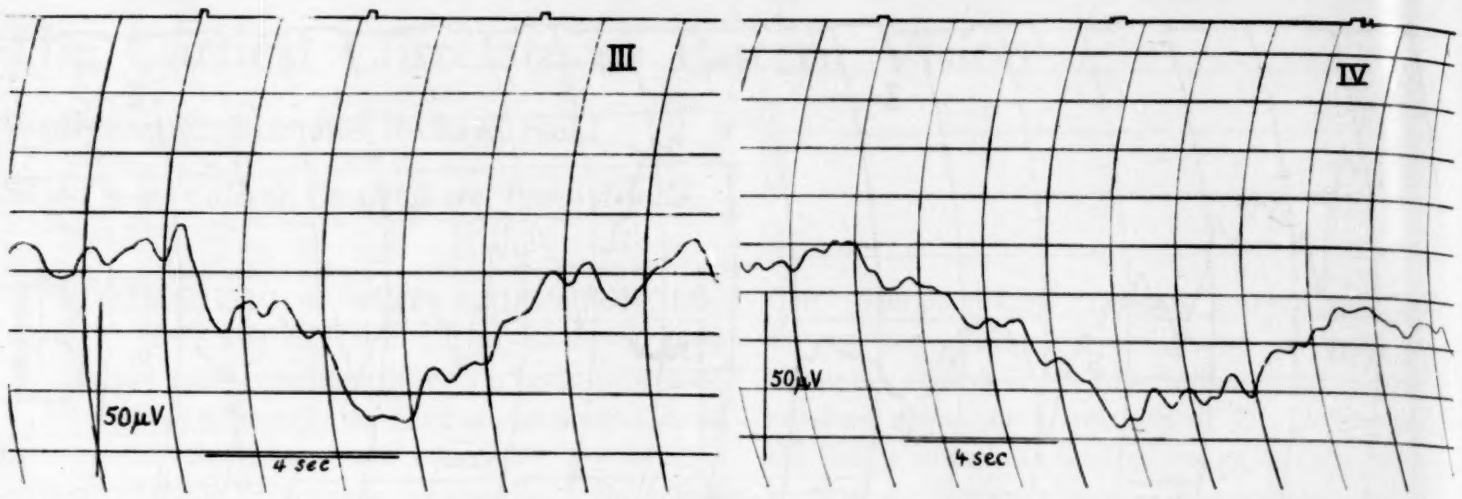

FIG. 2. Record III, subject M, one response to a longer exposure. Record IV, subject M, response to one exposure (12 sec).

again clearly discernible. They have the same polarity as those of Record I, and also almost the same size.

In Records I and II, responses give the impression of being short waves, merely because the records are written on very slowly moving paper and because the extension of the movement is restricted. Records III and IV (Fig. 2), taken from another subject, but with the same position of the electrodes, show reactions to single exposures of objects when the extension of the movement approximates 100° of visual angle, and when the paper of the recorder moves three times as fast as in Records I and II. In both records, there is an almost immediate response when the object begins its movement far in the periphery of the field (cf. the first mark on top). The deflection, which is again positive, grows as the object approaches the fixation mark; it becomes maximal near or at this point (second mark on top), and it gradually decreases as the object moves toward the end of its course (third mark). In Record IV, which is taken with a more slowly moving object, the total time of the exposure amounts to 12 sec. During this period, a current of constant polarity flows from the head into the amplifier. Its greatest intensity corresponds to about 40 μv. The fact that the deflection begins when the object moves at a distance of almost 50° from the fovea deserves particular attention. If such responses actually issue from the visual cortex, parts of this area seem to be interrelated by currents across very considerable distances.

Record V (Fig. 3), taken from a third subject, differs from Records I–IV mainly by the fact that the slowly responding galvanometer (of the recorder) used in the preceding experiments is now replaced by a much faster instrument. The response time of this instrument is .04 sec. As a result, Record V is covered with comparatively rapid oscillations, which the slower instrument could not register. The major positive deflection, which reaches its maximum (about 50 μv) at the fixation mark, is nevertheless perfectly obvious. In the present case, the response is restricted to the middle of the exposure (about 30° of visual angle). This happens occasionally, even if, with the same subjects, other responses extend from the beginning to the end of a wide exposure field. The variation may be related to differences in the attitude of the

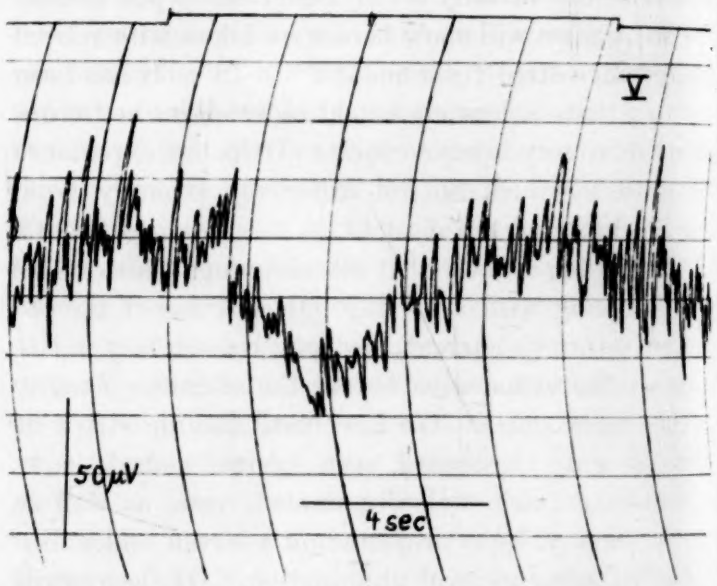

Fig. 3. Record V, subject R, response to one exposure, with alpha waves.

subjects who, while they fixate, may either concentrate on the fixation mark or follow the object with their attention.

When counting the faster oscillations in the periods before or after the exposure, one finds that their frequency is almost 10 cycles, the average frequency of alpha. In our experiments, this rhythm is never really suppressed when the visual environment remains more or less constant for some time. But changes, such as the movements of our experimental objects, tend to disturb the rhythm, at least for a while. Thus, in this record, the frequency, the amplitude, and the regularity of the oscillations appear to be affected during the exposure, particularly during its central part.

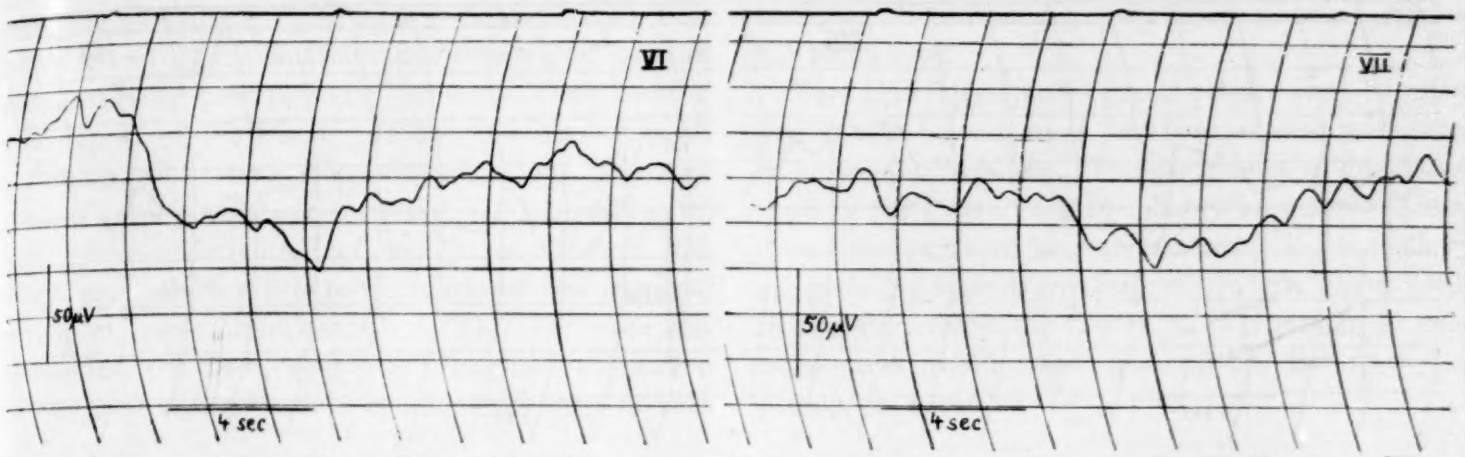

FIG. 4. Records VI and VII, subject M, asymmetrical responses with movements in opposite directions.

If the faster oscillations of Record V are alpha waves (or modified alpha), they represent, of course, one phase of what the cortex is actually doing while an object moves across the field. Strictly speaking, it would therefore seem desirable always to allow these oscillations to appear in our records, and thus to use a galvanometer, the response time of which is short enough for the purpose. We have nevertheless taken many records with our slower galvanometer (response time 0.5 sec), because the behavior of the slower potentials is sometimes more strikingly revealed when they are not covered with alpha. The following records, excepting XI, are taken with this instrument. After all, most records of alpha are taken under conditions which virtually eliminate the steady potentials of the brain. The fact that the slow instrument must also slightly distort steadier potentials can be ignored in a first exploration, which is not yet concerned with details.

Only a small central zone of the human retina seems to be represented in the parts of the visual cortex which lie, on both sides of the median fissure, directly under the skull. But localization within this zone is assumed to be specific; retinal cells on the left side of the central meridian are connected with cortical tissue in the left hemisphere, and cells on the right side with tissue in the right hemisphere. Just the opposite relations obtain, of course, between positions of outside objects and the locations of their cortical counterparts. We tried to decide whether the behavior of the currents here under investigation agrees with such facts. For this purpose, the occipital electrode was placed slightly to the right or the left of the median plane, while the second electrode remained attached to the region of the vertex. It seemed possible that the moving object would affect the occipital electrode more strongly when its cortical counterpart moved in the hemisphere corresponding to this electrode's position. We were thus led to the following four tests.

Records VI and VII (Fig. 4): In both tests the occipital electrode is placed on the right side of the median plane. With a movement of the object from left to right, i.e., retinally from right to left, the first half of the record ought to show a stronger response (VI). With the opposite direction of the movement, this asymmetry must be reversed (VII). Records VIII and IX (Fig. 5): In both tests the object moves left to right, i.e., retinally from right to left. With the occipital electrode on the left side of the median plane, the second half of the record must show a stronger response (VIII). With the electrode on the right side, the opposite must happen (IX). In such experiments, responses are expected to show a more specific shape, in spite of disturbances, and in spite of the fact that the impedance of the skull may vary from one part of the crucial region to another. We have nevertheless often obtained the predicted results. Records VI–IX may serve as examples. The asymmetries agree with our predictions.

One can go still farther in the same direction. If both electrodes are placed in the crucial occipital region, one on the left, and the other on the right side of the median plane, the cortical counterpart of the visual object will move first nearer one electrode, and then nearer the other. Under these circumstances, the resulting records ought to be diphasic; two deflections in opposite directions ought to follow each other. The difficulties inherent in such experiments are still greater than those involved in experiments VI–IX: visual potentials must be much weaker when the electrodes are placed so near each other, and responses will seldom appear to be convincingly diphasic if the curves in question show a major drift. Nonetheless, results are sometimes perfectly clear. For instance, in Records X and XI (Fig. 6), taken when the object moved from the right to the left, and when the left electrode was "active," a positive (downward) deflection during the first part of the exposure is followed by a negative deflection in the second half. With the connections used in our experiments, this is the expected sequence. The deflections are, of course, quite small (cf. the calibration).

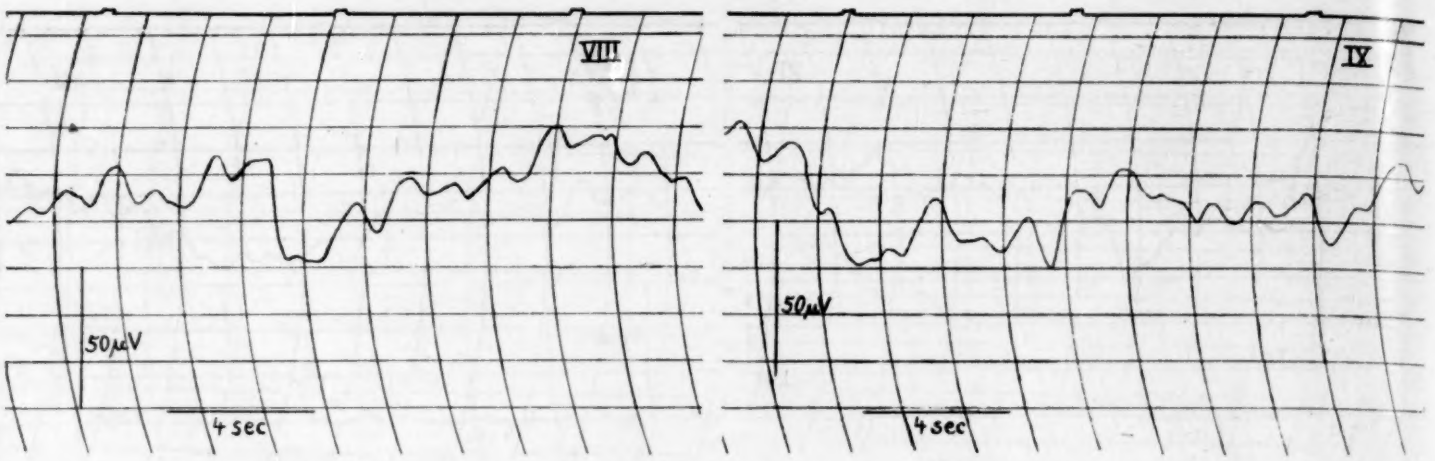

FIG. 5. Records VIII and IX, subject S, asymmetrical responses with different positions of the active electrode.

All our records have this in common: they seem to show that active parts of the visual area are surface-positive in relation to less active parts of the cortex; and that, therefore, when bright objects appear in the visual field the cortex is pervaded by currents which flow in corresponding directions. Libet and Kahn, Jr. recently obtained relatively steady potentials from other regions of the cat's cortex, but found no such responses in the visual area when the eyes were stimulated (*9*). We do not know what conditions of stimulation were used in these experiments. The negative result would contradict our findings only if the authors had also worked with moving objects of very moderate brightness.

In the meantime, the question arises whether our records are actually related to visual facts as such. It might, for instance, be suggested that such responses merely accompany eye movements, or that they represent psychogalvanic reflexes. Comparison with records obtained during eye movements and with the known characteristics of psychogalvanic reflexes has led us to the conclusion that these suggestions are not acceptable. Professor Gerard has called our attention to the possibility that vascular reactions might be associated with cortical fields. Obviously, this possibility will have to be examined; but, at the present time, the electric phase of vascular reactions does not seem to be sufficiently known for the purpose.

If the responses of our records should prove to be visual currents, our next question would refer to the origin of these currents in the visual system. As a radical answer, it might be held that responses such as these need not be interpreted in terms of steady potentials; when registered with a slowly reacting instrument, synchronized nerve impulses of considerable frequency would give similar curves. We hesitate to follow this interpretation. There is no question that, after Berger's discovery of alpha, numerous attempts have been made to find electric oscillations which accompany vision. The instruments used in such experiments have generally been well adapted to the purpose. If synchronized nerve impulses (or, rather, their fields) actually spread through skull and scalp while we see, the fact would probably have been discovered years ago. To be sure, further tests may be desirable.

It seems to us a more plausible assumption that the currents of our records represent the fields of impulses which are thoroughly nonsynchronized. It has been argued that if, from each part of a bright retinal image, nonsynchronized impulses arrive in a corre-

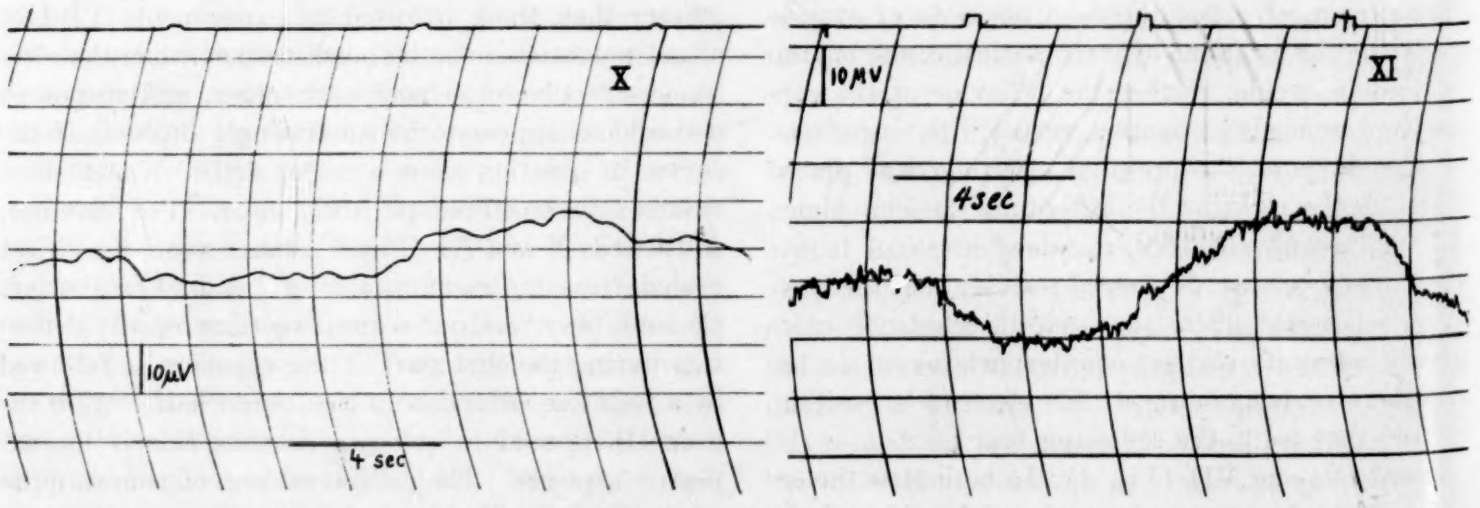

FIG. 6. Records X and XI, subjects M and E, diphasic responses.

sponding part of the visual cortex, the statistical result would be an approximately steady potential and an equally steady flow through and around the cortical counterpart of the image. If this is otherwise good reasoning, we have no cause for objection. The conclusion agrees with our own thesis. Elsewhere, we have given a derivation of steady potentials in the visual area, which refers to the much debated chemical action of nerve impulses (*5, 6, 7*). We need not choose between these two possibilities; the distribution of the resulting flow would be about the same in both cases, and this, from a psychological point of view, is the main issue.

More generally speaking, our results must be interpreted with some caution, just because they are related to important problems. The occurrence of direct currents in the cortex would probably have consequences in various parts of neurophysiology. In psychology, access to the cortical correlate of pattern vision would immediately affect the theory of psychophysical relations—and would affect first of all, the theory of perceptual space.

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TECHNICAL PAPERS

The Mutagenic Mode of Action of Formalin

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In 1946 Rapoport (*5*) reported that formalin, when mixed with the food of *Drosophila melanogaster* in sublethal concentrations, produces a high frequency of mutations. In the one experiment for which full data were given, the percentage of induced sex-linked lethals was 5.92. Kaplan (*2*) confirmed this finding, and similar results were obtained in this laboratory with a wild-type (OrK) stock. Rapoport attributes the mutagenic effect of formalin to a chemical reaction between the chromosomes and the CO group of the aldehyde. It seems rather unlikely that formalin as such would reach the chromosomes of the germ cells under the conditions of these experiments. Two other possibilities have to be considered. First, formalin might react with the food to produce a new mutagenic compound. Since formalin is added to the food while the agar is still well pourable, this reaction would take place at a temperature of about 60° C. Second, it also seems possible that a mutagenic substance is formed not in the food, but in the body of the fly either during digestion or in the germ cells.

In order to obtain information on this point, the effect of formaldehyde vapor on *Drosophila* was tested. A positive result with this method would prove that formaldehyde as such is a mutagen, which would make it at least probable that it is the effective agent in the feeding experiments also. A comparison of the effects of formaldehyde vapor on male and female germ cells in various stages of development should help to decide whether the action on the chromosomes is direct or mediated by the cytoplasm; for in the latter case female germ cells and spermatogonia should be more affected than mature spermatozoa. The OrK stock which had given 3–6% sex-linked lethals in previous feeding tests with formalin was used. Young adult ♂♂ and ♀♀ were exposed to formaldehyde vapor in the first series. Exposures lasted from 30 to 60 min, and the biologically effective dose was measured roughly by the survival rate during exposure and the first few hours afterwards. This does not take account of the fact that flies often die during the subsequent days. In all tests, ♀♀ survived in a larger proportion than ♂♂. In order to test germ cells which at the time of exposure had been at different stages of development, the treated ♂♂ were given fresh ♀♀ every fifth day, the treated ♀♀ were put on fresh food every fifth day, and mutation rates were recorded separately for the different broods. The untreated mates for both sexes were taken from the Muller-5 stock. Table 1 gives a short summary of the results.

The OrK stock used for these experiments has been tested repeatedly over the past few years. Sex-linked lethals in the ♂♂ arise at a fairly constant rate of about

0.3%. It is obvious that the treatment has remained ineffective, and that at doses which allow about 50% of the ♂♂ and about 75% of the ♀♀ to survive, formaldehyde neither reacts with the chromosomes directly to produce mutations nor with the cytoplasm of the germ cells to produce a mutagenic substance.

TABLE 1

THE EFFECT OF FORMALDEHYDE VAPOR ON MUTATION RATE IN GERM CELLS OF IMAGINES

Sex	Survival rate	No. of tested X-chromosomes	No. of lethals	Percent of lethals
	43/60	1172	1	
♂♂	7/24	451	0	
	13/28	983	3	
		2606	4	0.2
♀♀	22/28	1136	0	
	21/28	1342	1	
		2478	1	0.05

Since it seems legitimate to consider the experiments as tests for spontaneous lethals, an interesting incidental result consists in the confirmation of two previously reported features of the spontaneous mutation rate. First, the mutation rate is higher in ♂♂ than in ♀♀ (*1*). Second, when the mutation rate is recorded separately for the four successive broods from treated ♂♂, the following figures are obtained: first brood, three lethals in 860 chromosomes; second and third broods, no lethals in 1543 chromosomes; fourth brood, one lethal in 203 chromosomes. This curve of mutation rate with a peak for the first sperm used and an increase for spermatozoa which have been stored for a considerable time, has been described by Lamy (*3*), and Muller (*4*).

As the feeding method only treats larvae, the negative result obtained on adults did not seem conclusive. Additional tests on larvae were carried out with a new apparatus which allowed milder and therefore longer exposures to be made. In the first experiments old larvae were used, because these can stand a fairly long exposure. However, when feeding tests, carried out at the same time, indicated a sensitive period to the mutagen early in the third instar, tests were also carried out on younger larvae of this age. These larvae can stand only a much shorter exposure without being killed or sterilized.

TABLE 2

THE EFFECT OF FORMALDEHYDE VAPOR ON MUTATION RATE IN THE GERM CELLS OF LARVAE

Time in hr after laying of eggs	Length of exposure	No. of tested X-chromosomes	Lethals	
			No.	%
96–100	2 hr	2126	2	0.1
about 70	2 hr	138	0	0
46–56	2 hr	169	1	
	70 min	408	1	
	50 min	436	1	
		1013	3	0.3

In all tests, a considerable proportion of the larvae died either during exposure or later on. The two sexes had the same survival rate. Table 2 summarizes the data gained from exposure of larvae.

In all series, the results are negative. Thus, all attempts to induce mutations by formaldehyde vapor have failed, and it seems most likely that the effective mutagen is a compound formed by reaction of formaldehyde with one of the components of the food. Experiments to identify this component have been started.

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Inhibition of Phosphatases by Beryllium and Antagonism of the Inhibition by Manganese

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Beryllium poisoning has become a subject of considerable practical importance as a result of the use of beryllium compounds in the fluorescent light industry and in several other industrial processes. The occurrence of a number of cases of beryllium poisoning as a result of the industrial use of this metal has emphasized the necessity of obtaining detailed information on the toxicity and mode of action of beryllium in mammals. While numerous studies have been carried out on the toxicology of beryllium, little is known of its mechanism of action and no therapeutic measures for either acute or chronic beryllium poisoning are available.

In undertaking studies on the mechanism of action of beryllium, we were interested in examining its effect on enzymatic reactions with special attention being directed toward enzymes requiring metallic activators. The occurrence of beryllium in the same atomic group with calcium and magnesium and the many similarities in the chemical behavior of these three metals suggested that the toxic effects of beryllium might involve interference with the biological functions of calcium and magnesium. The present communication describes the results of experiments which demonstrate that beryllium inhibits alkaline phosphatases activated by magnesium and calcium, and that this inhibition can be prevented and re-

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versed by manganese.

In order to ascertain the action of beryllium on a calcium-activated enzyme, the adenosine triphosphatase system was employed, and the effect of beryllium on the adenosine triphosphatase activity of mouse liver and lung tissue was measured by the method of DuBois and Potter

TABLE 1

THE EFFECT OF BERYLLIUM ON THE ATP-ASE ACTIVITY OF MOUSE LIVER AND LUNG TISSUE

Tissue	Molar concn. of beryllium	ATP-ase units	% Inhibition
Liver	0	13.4	..
	6.1×10^{-3}	1.0	92
	3.0×10^{-3}	3.0	78
	8.0×10^{-4}	9.3	21
Lung	0	17.3	..
	6.1×10^{-3}	1.7	90
	3.0×10^{-3}	4.4	74
	8.0×10^{-4}	10.8	37

(*2*). For all of our tests, aqueous solutions of beryllium chloride were employed. The inhibitory action of beryllium on adenosine triphosphatase activity is shown by the data in Table 1, from which it may be seen that the activity of lung and liver tissue was inhibited to about the same extent by similar concentrations of beryllium.

TABLE 2

THE INHIBITORY ACTION OF BERYLLIUM ON ALKALINE PHOSPHATASE OF RAT TISSUES

Tissue	Phosphatase activity mg P/g tissue/hr	Molar concn. of Be for 50% inhibition
Serum	0.33	1.8×10^{-6}
Duodenum	34.20	5.0×10^{-6}
Kidney	32.6	5.9×10^{-5}
Lung	1.78	4.5×10^{-4}
Brain	0.72	5.9×10^{-4}

In view of the inhibitory action of beryllium on adenosine triphosphatase activity, we were interested in testing the effect of beryllium on magnesium-activated phosphatases. The similarity in the chemical behavior of magnesium and beryllium was first observed in 1888 by Sestini (*5*) who found that beryllium could replace magnesium in the growth of plants. Recently Grier, Hood, and Hoagland (*3*) and Klemperer, Miller, and Hill (*4*) have demonstrated that beryllium exerts a strong inhibitory action on alkaline phosphatases. To compare the inhibitory action of beryllium on phosphatases activated by magnesium and calcium, we have examined the effect of beryllium on the alkaline phosphatase activity of several tissues. Alkaline phosphatase measurements were performed using essentially the method of Bodansky (*1*) with modifications in the quantities of reactants to make the method suitable for small quantities of tissue. The test system contained 1 ml of 0.025 M Veronal buffer (pH 8.9) containing 0.015 M sodium β-glycerophosphate, 1–100 mg of homogenized tissue or serum, and enough water to make a final volume of 1.7 ml. Solutions of the various metals employed replaced an equivalent amount of water in the test system. After incubation for 30 min at 38° C, the reaction was stopped by the addition of 0.3 ml of 50% trichloracetic acid and after centrifugation the inorganic phosphate liberated was measured. Table 2 gives the alkaline phosphatase activity of several tissues in the absence of added metallic ions. The values are expressed as mg of phosphorus liberated per g of tissue per hr together with the molar concentration of beryllium necessary to produce 50% inhibition of the alkaline phosphatase activity of each tissue. These results demonstrated the pronounced inhibitory action of beryllium on magnesium-activated phosphatases. There was a considerable difference in the sensitivity of the alkaline phosphatase of serum and various other tissues to beryllium, with serum phosphatases being the most sensitive of those studied.

TABLE 3

ANTAGONISTIC ACTION OF MANGANESE ON THE INHIBITION OF SERUM PHOSPHATASE BY BERYLLIUM (5.9×10^{-10} M)

Molar concn. of manganese	% Inhibition
0	77
5.9×10^{-3}	4
5.9×10^{-4}	13
2.9×10^{-4}	51
5.8×10^{-5}	64

It is likely that the inhibitory action of beryllium on phosphatases results from competition with magnesium and calcium for the same group on the enzyme with which they react. In this connection we were interested in ascertaining whether increasing the concentration of magnesium or addition of some other metallic ion to the system would decrease the amount of inhibition of serum phosphatase by beryllium. Various concentrations of magnesium as high as 5.9×10^{-3} M did not influence the inhibitory action of beryllium. This observation was in agreement with the results of other investigators (*3*). This indicated that beryllium has a much greater affinity for the enzyme than does magnesium. Since manganese also activates alkaline phosphatases, we tested the inhibitory action of beryllium on serum phosphatase in the presence of various concentrations of manganese as manganese sulfate. When manganese and beryllium were present in the test system at the time of addition of the serum, manganese markedly decreased the inhibition produced by beryllium, as illustrated by the data in Table 3. It was also found that manganese was effective in antagonizing the action of beryllium when added to the reaction mixture 5 min after the beryllium and serum were mixed. Cobalt and nickel also antagonized the inhibitory effect of beryllium on serum phosphatase *in vitro*.

The results of these experiments give support to the hypothesis that beryllium interferes in biological reactions in which magnesium and calcium participate. Magnesium-activated reactions appear to be more sensitive toward the action of beryllium than calcium-acti-

vated reactions. The extent to which the effects of beryllium on phosphatases are involved in acute and chronic beryllium poisoning must be determined by further experimentation on beryllium-poisoned animals. The ability of manganese to counteract the inhibitory effects of beryllium on phosphatases *in vitro* is encouraging because it provides evidence of the possibility of reversing the combination of beryllium with tissue constituents and thereby suggests a possible approach to the development of therapy for beryllium poisoning. In this connection, experiments are in progress at the present time in which the effects of several metals on acute and chronic beryllium poisoning are being tested. The results of these experiments will be reported in detail elsewhere.

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The Skin Temperature of an Extremity as a Measure of Its Blood Flow

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Skin temperature often is used as an index of blood flow in the extremities, even though the relation may be merely implicit. The purpose of this communication is to emphasize the fact, mentioned by Lewis (*3*) and others, that skin temperature is valid as a measure of extremity blood flow under certain conditions only.

Skin temperature depends upon both the rate of heat supply to the skin and the rate of heat removal—that is, upon the temperature and flow rate of the blood, and the insulation and temperature difference between skin and environment. If the temperature difference is small, heat will be removed so slowly that even large changes of blood flow will have little effect on the skin temperature. This is illustrated in the figures, in which are plotted finger blood flow and finger temperature against time. (Finger blood flow was measured with the plethysmographic method of Goetz (*2*) using air transmission. The values given are the averages of the flow at systole and at the end of diastole. Finger temperatures were measured with small thermocouples attached with adhesive tape, and connected to an instrument sensitive to 0.15° C and recording at 80-sec intervals. The hand was bare.) At a room temperature of approximately 32° C it is obvious from Fig. 1 that the finger surface temperature bears no relation to changes of blood flow. The correspondence is scarcely improved at an ambient temperature of about 21° C (Fig. 2). The air movement in both these experiments was the same.

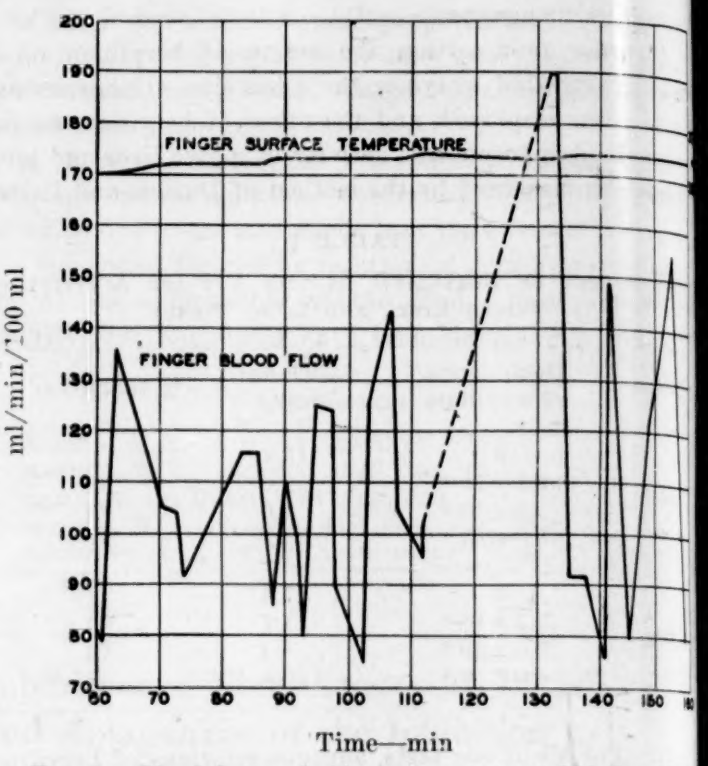

Fig. 1. Blood flow and skin temperature at 32° C ambient. Hand air insulation = 0.54 equivalent clo.

At about 7° C room temperature, with greater air movement, skin temperature follows the blood flow pattern fairly well, as can be seen in the example given in Fig. 3. At −34° C, with a gradient of 55–66° C between the bare hand and the ambient air, the lag between blood flow change and skin temperature change is probably negligible; for example, a very minor emotional disturbance may cause a fall of finger temperature of 8° C within 2 min; or a few deep breaths, of 3° C.

Our experience, exemplified by the figures, is that the heat loss rate calculated for the hand should be 12 kg-cal/hr (240 kg-cal/hr/m²) or more, if the skin tempera-

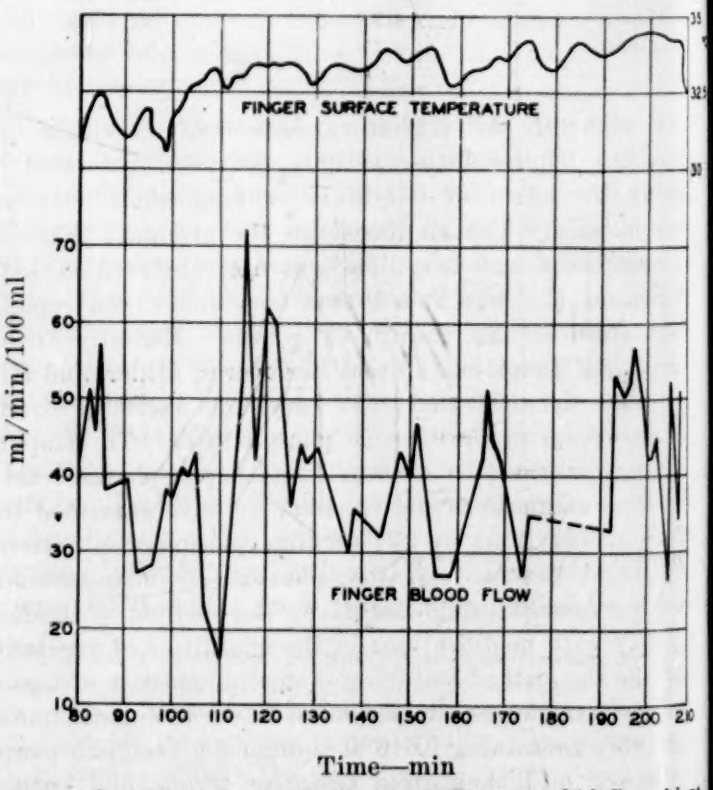

Fig. 2. Blood flow and skin temperature at 21° C ambient. Hand air insulation = 0.54 equivalent clo.

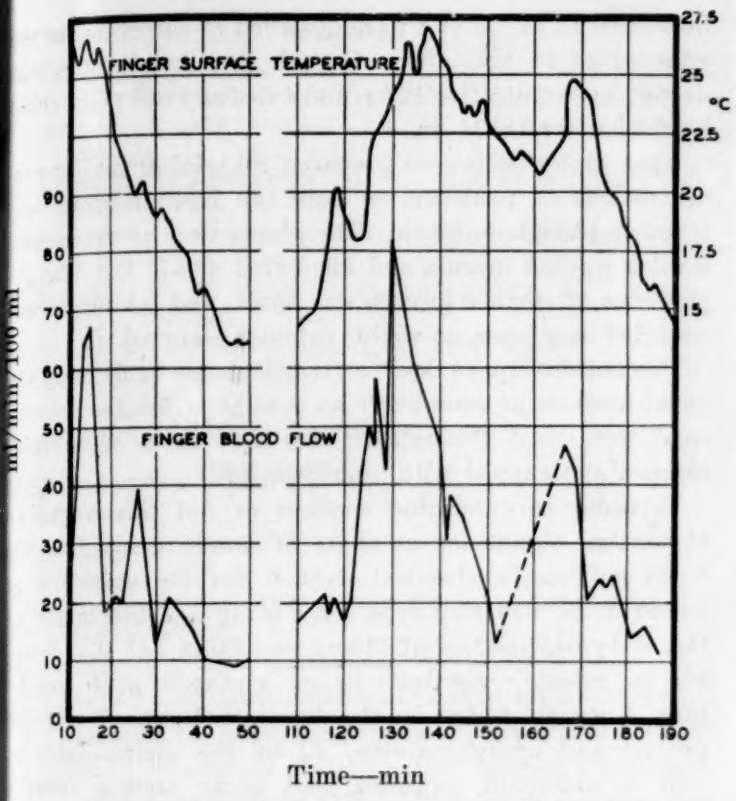

FIG. 3. Blood flow and skin temperature at 7° C ambient. Hand air insulation = 0.30 equivalent clo.

ture is to follow closely the blood flow changes. It is likely that this approximate minimum applies to other areas of the body. This heat loss rate corresponds, for example, to an air insulation over the bare hand of 0.54 equivalent clo[1] (insulation unit) and a temperature difference of 24° C between hand and air; or to a temperature difference of 13° C if the air insulation is 0.30 equivalent clo.[2] Thus, if the temperature of the hand is expected to fall to 21° C the ambient temperature should be −3° C; or 8° C for the higher air movement. If only gross, slow changes of blood flow are to be measured, correspondingly lower heat loss rates may be adequate.

Good blood flow rates may be achieved in normal persons even with high gradients, provided that only the part under investigation is exposed to the cold, and the rest of the body is warm. This can be done by insertion of, say, the extremity of a nude subject into a cold box in a warm room; or by exposure to a cold room of the extremity of a warmly dressed subject. It is true that minute variations of temperature can be measured with high sensitivity apparatus, and that these, with some lag, might represent blood flow more exactly than do the examples given. Such a technique has serious drawbacks, and it would appear advantageous to apply the laws of heat transmission in order to amplify and accelerate temperature changes.

In summary, if direct measurement of blood flow is not feasible, and skin temperature is used instead, the part of the body under investigation must lose heat at a rate of more than about 240 kg-cal/hr/m² for good correspondence between blood flow and skin temperature changes. If local cold stimulus must be avoided, a flow method must be employed.

[1] The clo equation is not strictly applicable to a portion of the body, having been derived for the body as a whole (*1*).

[2] Recent determinations with an electrically heated manikin give an insulation of the air around the hand of 0.54 equivalent clo when that of the whole body is 0.82 clo; and of 0.30 equivalent clo when the average for the body is 0.43 clo.

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The Action of Penicillin on *Bacillus subtilis* Growing in the Absence of Amino Acids[1]

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Gale and his co-workers (*2*) have reported that Gram-positive bacteria are able to assimilate glutamic acid from the medium in which they are grown and to concentrate the free amino acid within the bacterial cell. Gram-negative organisms, on the other hand, appear to be unable to do so. They further observed that when certain strains of *Streptococcus faecalis* and *Staphylococcus aureus* were exposed to penicillin during the logarithmic phase of growth, the ability to concentrate free glutamic acid in the resting cell was lost. Subsequently Bellamy and Klimek (*1*) noted that a strain of *Staphylococcus aureus* which had been trained to grow in extremely high concentrations of penicillin changed from its usual morphology to that of a Gram-negative coccobacillus and acquired the ability to grow in a medium containing no amino acids. Gale (*2*, *3*) also found that a strain of *Staphylococcus aureus* trained to grow in a medium deficient in amino acids, acquired *pari passu* a considerable degree of penicillin resistance. He therefore suggested that "penicillin interferes with the mechanism whereby certain amino acids are taken into the cell, and that the sensitivity of the cell to penicillin is then determined by the degree to which its growth processes are dependent upon assimilation of preformed amino acids rather than upon their synthesis."

At least two objections may be raised to this hypothesis. In the first place, some of the Gram-negative coliform organisms are inhibited by high concentrations of penicillin, and yet most of them require no amino acids for growth (*7*). Secondly, *B. subtilis*, a Gram-positive organism with many strains sensitive to penicillin, has been reported in some instances to grow in media containing only ammonia as a source of nitrogen (*5*).

A search was therefore made for strains of *B. subtilis* which would grow in a synthetic medium devoid of amino acids in order to determine whether such strains would be inhibited by penicillin.

[1] This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

The first few strains investigated grew readily in various synthetic media deficient in amino acids, and under certain conditions were completely inhibited by penicillin. Irregular results were obtained, however, and it eventually became apparent that these could be attributed to the production of penicillinase by the organisms.

A strain of *B. subtilis* (PCI-220-NNRL-B-558) (*6*) was finally obtained which did not produce penicillinase and which grew readily in a synthetic medium, "Base B" (Table 1), containing only ammonium sulfate as a source of nitrogen. In this medium the organism was just as sensitive to penicillin as it was in a "complete" medium (tryptose phosphate broth) irrespective of wide changes in the age of the culture, the size of the inoculum, and the time of reading. (See Table 1.)

TABLE 1

PENICILLIN SENSITIVITY OF *Bacillus subtilis* (PCI-220-NNRL-B-558)

Medium	Inoculum 18-24-hr culture in B.B.		Time of reading in hr	Inhibiting concentration of penicillin u/ml
	Amount and dilution	Approximate no. of viable organisms		
T.P.* broth ..	.2ml 10^{-1}	2×10^4	18	.008
			90	.008
	.2ml 10^{-5} (unwashed)	20	18	.008
			90	.008
B.B.†	.2ml 10^{-1}	10^3	20	.008
			96	.008
	.2ml 10^{-4} (washed)	10	20	.008
			96	.008
T.P. agar	.2ml 10^{-1}	2×10^4	18	.01
			90	.05
	.2ml 10^{-4} (washed)	6×10^2	18	.01
			96	.01
B.B. agar	.2ml 10^{-1}	10^3	20	.01
			96	.01
	.2ml 10^{-4} (washed)	10	20	.01
			96	.01

* Tryptose phosphate.

† "Base B," composition as follows, wt/liter of medium: KH_2PO_4 – 4.5 g; NaOH – 1.1 g; Fe So_4 – $7H_2O$ – 0.0139 g; Mg SO_4 (anhyd.) – 0.02 g; $(NH_4)_2SO_4$ – 0.73 g; glucose – 2.25 g (autoclaved separately); sodium thioglycolate – 0.27 g; thiamine (subsequently found to be unnecessary for growth) – 0.00003 g (autoclaved separately); nicotinic acid (subsequently found to be unnecessary for growth) – 0.001 g; distilled H_2O to 1000 ml. Adjusted to pH 7.4.

Determinations of sensitivity to penicillin were carried out in fluid media as follows: A known quantity of crystalline sodium penicillin G was dissolved directly in the basal medium to give an appropriate concentration. Serial dilutions were then made in 1 ml volumes so that eight tubes, containing concentrations of penicillin varying from 0.15 u/ml to approximately 0.001 u/ml, were obtained. All tubes including a control were inoculated with 0.2 ml of a culture which had been washed three times in sterile saline and resuspended in the basal medium immediately before inoculation. The tubes were incubated at 37° C and were observed at intervals for the appearance of turbidity. Growth in control tubes was usually apparent after 12 hr, and a distinct end point could be read after 18–24 hr.

Agar plates[2] were also prepared containing similar concentrations of penicillin in both the basal medium and tryptose phosphate broth. The plates were streaked with similar washed inocula and incubated at 37° C. The appearance of surface growth was noted, and inhibition was recorded only when no visible colonies occurred.

Precautions were taken to exclude amino acids from the basal medium as completely as possible. Reagent chemicals[3] were used throughout, and test tubes were acid-cleaned and capped with aluminum foil.

In order to determine whether or not this organism elaborated significant amounts of amino acids into the basal medium, a chemical method for the detection of amino acids was employed. By using a modification of the ninhydrin method of Moore and Stein (*4*) it is possible to detect concentrations of glutamic acid smaller than 1 μg/ml added to the basal medium. Excepting proline and hydroxyproline, all of the amino acids as well as ammonia, peptides, and many amines form a colored product with ninhydrin showing an absorption peak at 570 mμ. If the basal medium is aerated for 5 hr at pH 11.5, most of the ammonia is driven off. After neutralization the solution may then be treated with ninhydrin and the color measured with a spectrophotometer at 570 mμ. When this method was employed, the basal medium, thought to be free of amino acids, was found to give a blank value equivalent to that obtained with 0.5–1.0 μg/ml of glutamic acid. It seems likely that most of this color was actually due to residual ammonia, and in any event the value obtained sets an upper limit for possible traces of amino acids in the medium.

In an experiment in which a washed inoculum of this strain of *B. subtilis* was grown in the basal medium for 24 hr, samples of the culture were removed at 6-hr intervals for the following determinations: turbidimetric reading, dry weight of organisms, count of viable organisms, and concentration of ninhydrin-reacting substances in the aerated filtrate. During the first 12 hr, when maximum growth took place, there was no demonstrable increase in ninhydrin-reacting substances in the filtrate, although at 18 and 24 hr, with slowing of growth and death of organisms, small amounts appeared.

After 18-hr incubation there were 200 μg of organisms by dry weight in 1 ml of culture. Since there was no more than 1 μg of amino acid/ml present in the basal medium originally, and since dried bacteria contain an average of 60% protein (*5*), it is apparent that less than 1% of the protein synthesized during growth could be accounted for by amino acids assimilated from the medium.

It is concluded, therefore, that penicillin inhibits the growth of this particular strain of *B. subtilis* by some mechanism other than interference with the assimilation of preformed amino acids by the bacterial cell.

[2] The agar was washed 5 times overnight in distilled water.

[3] Except for Difco sodium thioglycolate.

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A Source of Error in Tracer Experiments with P^{32} [1]

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In dealing with radioactive solutions of high specific activity where total phosphorus is small, the amount of P^{32} adsorbed on laboratory glassware may become significant.

An experiment done in this laboratory to measure this adsorption and to determine the efficacy of silicone-coated glassware illustrates the magnitude of the problem.

A solution of P^{32} containing about 1×10^{-4} mg /ml of total phosphorus was drawn up once into each of four 2-ml pipettes, all new and identical except that two had been coated with silicone. Each of the pipettes was then washed by drawing up 2-ml amounts of distilled water. The wash water from each pipette was collected into two counting sample cups (1 ml each), evaporated, and the number of radioactive disintegrations determined in a Nucleometer.

Average values, in cpm/ml were:

	Coated	Uncoated
Wash 1	390	6,000
Wash 2	110	300
Wash 3	background	110

The count in the first wash of the uncoated pipette is high and variable, depending on both the P^{32} and P^{31} contents, and can cause serious error where it is not suspected.

To avoid this difficulty, we have adopted several techniques. Wherever possible all counting solutions contain a uniform 1 mg/ml of carrier phosphorus at pH 4.5. Where less carrier must be used, care is taken to pipette as rarely as possible and only in solutions containing as much carrier as the procedure permits. When conditions are extremely unfavorable, requiring exceedingly low carrier or high pH values, silicone coating of glassware is necessary.

The coating technique we used was modified from Johannson and Torek (*1*). A 2% solution of "DC 200" was prepared in pure grade CCl_4 (the Dow Company recommends chlorinated organic solvents). The glassware, preferably new, is scrupulously cleaned and heat-dried, completely immersed in the silicone solution, and carefully drained. It is then placed into an oven at 90° C for 2 hr, to evaporate the solvent. The temperature is then raised to 250° C for a period exceeding 4 hr but not longer than overnight. Detailed experiments on the lasting qualities of this type of coating have been done by the Dow Company. This treatment caused no detectable change in calibration.

There is another group of compounds which have been similarly used, called "silanes" (hydrochlorides of unpolymerized silicones). These may be vaporized onto a glass surface, where they react with the film of moisture to form a silicone layer. This process of hydrolysis and polymerization is somewhat difficult to control.

[1] Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.

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Free Amino Acids and Peptides in Frog Embryos

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Relatively large quantities of free amino acids have been detected by two-dimensional paper chromatography in tissues of the mouse (*3*, *4*), the human, and the frog (*Rana pipiens*). Each of the normal adult tissues examined appeared to have a distribution of free amino acids characteristic for that tissue. Therefore, it was apparent that the ability of these tissues to maintain the characteristic distributions of these constituents must have arisen at some time during embryonic or postembryonic development.

As the first step in the study of the free amino acids of frog tissues at various stages of development, examinations were made of alcohol extracts of material ranging from ovarian eggs to larvae, corresponding to stages 0, 1, 2, 8, 10, 12, 17, and 21 according to Shumway (*5*). In the last stage examined, there was still no intake of food from the environment. Exactly 260 mg of fresh weight of material was placed in ethyl alcohol, the final concentration of alcohol being 70–73%. The material was homogenized in a ground glass homogenizer, centrifuged, and the supernatant fluid decanted and evaporated to dryness. The residue was thoroughly extracted with 3 ml of distilled water. This was then dialyzed against 10 ml of distilled water in a rocking dialyzer (*2*). One half of the dialyzate was evaporated to dryness and placed on paper for chromatography (*1*), and the other half was chromatographed after hydrolysis with 6 N HCl.

In none of the stages studied were there detectable quantities of free amino acids in the extracts. Several ninhydrin-reactive materials were located at spots which disappeared on acid hydrolysis, and which did not correspond in position to any known amino acids. These were

probably peptides which gave rise to the small quantities of aspartic acid, glutamic acid, glycine, and alanine which were detected in most of the samples after acid hydrolysis. The quantities of glutamic acid were greater than those of the other amino acids in the hydrolyzates.

These results indicate clearly that the ability to maintain the relatively high content of intracellular amino acids of the adult frog must appear at some time after the last stage of development examined in the present study.

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The Interaction of Antimalarials with Nucleic Acids[1]

I. Acridines

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II. Quinolines

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Department of Physiological Chemistry,
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From studies of the physicochemical factors which are of possible importance in the therapeutic activity of antimalarials, we have secured data on the ionization exponents of quinoline and acridine derivatives (*8–10*), and have reported briefly on the interaction of these compounds with plasma proteins (*11*). The present preliminary report on the interaction of these agents with nucleic acids is of interest not only from the standpoint of the possible relationship to antimalarial activity, but also because such interactions provide a plausible basis for explaining the effect of ribonucleic acid in reversing the inhibition by acridines of the growth of certain bacteria (*12, 13*), yeast (*14*), and bacterial viruses (*5, 6*). In addition, these studies provide procedures which may be useful in investigating the structure of nucleic acids and the action of the nucleases.

Yeast ribonucleic acid from the Schwarz Laboratories was purified by the procedure of Fletcher *et al.* (*7*) and Vischer and Chargaff (*19*), particular attention being paid to removal of inorganic salts by dialysis because of the large effect of ionic strength upon the interaction. Microanalysis of a sample dried at 110° C gave: N (Dumas) 16.1%; P (Pregl-Lieb) 8.8%.

[1] Supported in part by a grant from the Penrose Fund of the American Philosophical Society.

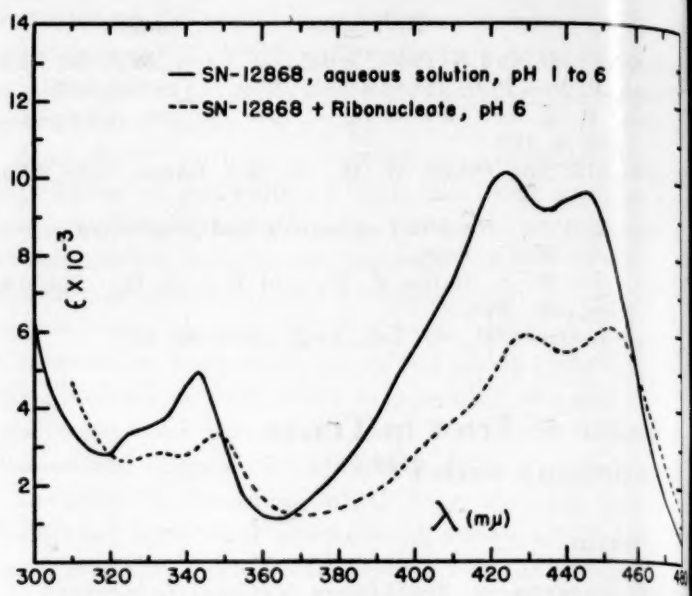

FIG. 1. Absorption spectra for SN-12868.

In Fig. 1, absorption spectra are presented for 2-methoxy-6-chloro-9-(1′methyl-8′-diethylamino-octylamino)-acridine (SN-12868)[2] in aqueous solutions buffered with phosphate at pH 6.0 ($\Gamma/2 = 0.1$) in the absence of and in the presence of sodium ribonucleate at a concentration (0.5 g/100 ml) at which the change in absorption is complete. Half-maximum transformation at this pH and ionic strength is attained when the concentration of ribonucleate (calculated as the anhydrous acid) is 0.013 g/100 ml. The interaction is stronger at lower ionic strength, and is somewhat stronger at pH 6.4 than at pH 6.0. Similar changes in spectrophotometric absorption in the presence of nucleates are obtained with 7-chloro-4-(1′-methyl-4′-diethylaminobutylamino)-quinoline (SN-7618). That the change in absorption is an indication of some type of bond between the nucleate anion and the antimalarial has been verified in the case of the acridine by determining the effect of sodium ribonucleate on the distribution of the acridine between organic solvents and buffered aqueous solutions.

In order to evaluate the interaction more exactly, we have used an adaptation of the mathematical treatment of the ionization of polyvalent acids proposed by Simms (*18*) and by von Muralt (*16*). A maximum number, m, of quinoline or acridine ligands can combine with one molecule of nucleic acid, the nucleic acid being present in a total molar concentration, T^*. For the special case in which the interacting groups in the polyvalent molecule are identical, and electrostatic interference between successively bound ligands is negligible, the binding reaction can be treated as if it consisted of the combination of one molecule of ligand per molecule of monovalent nucleic acid of total molar concentration, T, with $T = mT^*$. The process is represented as

$$N + L \rightleftharpoons NL \qquad (1)$$

An association constant, k', is defined by the equation

$$k' = \frac{[NL]}{[N][L]} \qquad (2)$$

[2] These are the code numbers employed in the publication *A survey of antimalarial drugs, 1941–1945*, F. Y. Wiselogle, Ann Arbor, 1947.

in which brackets are used to represent molar concentrations at equilibrium, N is the free monovalent nucleic acid, L is the free ligand, and NL is the complex consisting of one monomeric molecule of ligand combined with one reacting center on the nucleic acid, this center being considered as if it were a separate monovalent molecule. The constant, k', is the intrinsic constant for the special case defined above. This constant can be evaluated spectrophotometrically essentially as described by Clark *et al.* (*1*, *17*) for the interaction of nitrogenous bases with metalloporphyrins. The equation is:

$$-\log [N] = -\log \left[T - \frac{S(D_1 - D)}{(D_1 - D_2)} \right] = \log k' + \log \frac{D - D_2}{D_1 - D} \qquad (3)$$

in which S is the molar concentration of total ligand which is kept constant during the series of measurements; D_1 is the optical density of the solution when nucleic acid is absent, D_2 is the optical density when the concentration of nucleic acid is sufficiently large to cause complete binding of the ligand, and D is the optical density at some intermediate point at which both species L and NL are present.

Inasmuch as Michaelis and Granick (*15*) have suggested that the polymerized forms rather than the monomeric species of certain basic dyes, such as methylene blue, interact with polyvalent anions of the chondroitin sulfate type (but presumably not with nucleate anions according to these authors), it was desirable to formulate the possible combination of N with L_2, the dimeric species of ligand. The processes would be:

$$L_2 \rightleftharpoons 2L\,;\; N + 2L \rightleftharpoons NL_2 \qquad (4)$$

An association constant for the latter process can be expressed as:

$$k' = \frac{[NL_2]}{[N][L]^2} \qquad (5)$$

$$-\log [N] = -\log \left[T - \frac{S}{2}\left(\frac{D_1 - D}{D_1 - D_2}\right) \right] = \log k' + \log \frac{2S(D - D_2)^2}{(D_1 - D)(D_1 - D_2)} \qquad (6)$$

In order to compare the data for SN-12868 with that for SN-7618 on the same graph, it is convenient to define a degree of dissociation, $\alpha \equiv [L]/S$. Equations (3) and (6) then take the form of (7) and (8), respectively:

$$-\log [N] = -\log [T - S(1 - \alpha)] = \log k' + \log \frac{\alpha}{1 - \alpha} \qquad (7)$$

$$-\log [N] = -\log \left[T - \frac{S}{2}(1 - \alpha) \right] = \log k' + \log \frac{2S\alpha^2}{1 - \alpha} \qquad (8)$$

The values of T can be calculated for each equilibrium position provided that the molecular weight of the nucleic acid (from which T^* is calculated) and the value of m are known since $T = mT^*$. The molecular weight of the preparation which we have used is unknown. Nevertheless, valuable preliminary tests of the relationships given above can be made by expressing the total concentration of nucleic acid in terms of the gram-atoms of nucleic acid phosphorus per liter of solution (P). The values of T^* and T are related to P by the equation:

$$T = mT^* = mP/p \qquad (9)$$

in which p is the number of phosphorus atoms per molecule of nucleic acid. Although absolute values of m and p cannot be calculated when the molecular weight is unknown, the ratio, m/p, can be determined from the spectrophotometric data and the percentage of phosphorus in the nucleic acid.

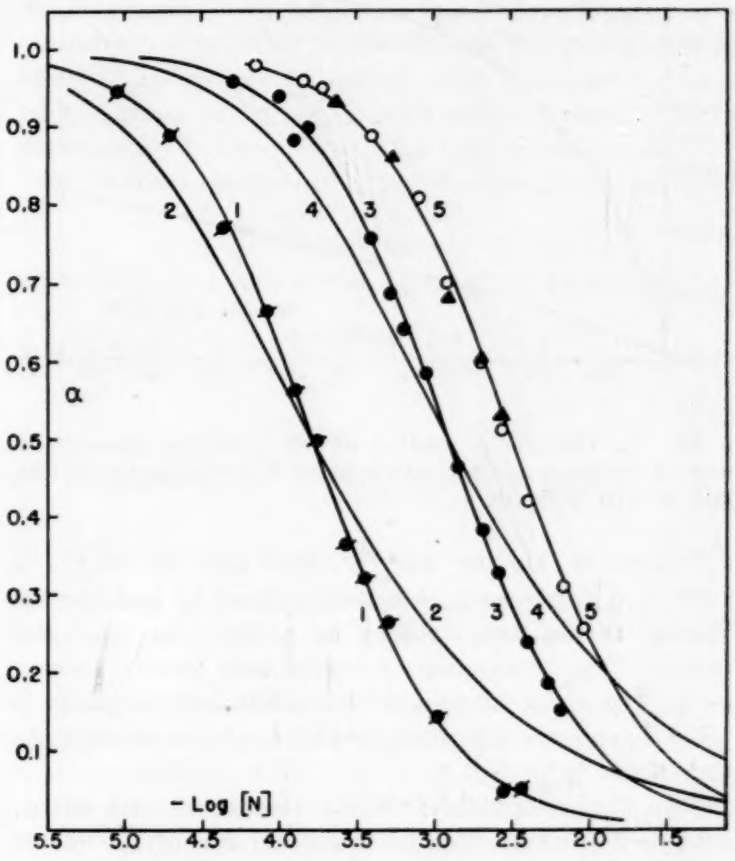

FIG. 2. Interaction of antimalarials with sodium ribonucleate at pH 5.85. -●- SN-12868: $\Gamma/2 = 0.1$; ○ SN-12868: $\Gamma/2 = 1.05$ with sodium chloride; ▲ SN-12868: $\Gamma/2 = 1.06$ with magnesium sulfate; ● SN-7618: $\Gamma/2 = 0.1$. Theoretical curves drawn according to equation (7): curve 1, log $k' = 3.79$; curve 3, log $k' = 2.91$; curve 5, log $k' = 2.50$. Theoretical curves drawn according to equation (8): curve 2, log $k' = 8.19$, $S = 4 \times 10^{-5}$ M; curve 4, log $k' = 7.21$, $S = 5 \times 10^{-5}$ M.

In Fig. 2, data for the interaction of SN-12868 and SN-7618 with yeast ribonucleate are compared with theoretical curves drawn according to equations (7) and (8); the values of the constants are given in the legend of this figure. The values for these constants are tentative pending further work with nucleic acid preparations of proven homogeneity and known molecular weight. However, the present data permit comparison between the relative strengths of interaction of the quinoline and acridine derivatives. In both cases the interactions appear to correspond to the combination of monomeric rather than dimeric ligand with the nucleic acid. The constant for the binding of SN-12868 with the nucleic acid is approximately 7.6 times that of SN-7618 at the same pH and ionic strength.

Increase in ionic strength from 0.1 to 1.05, either by addition of sodium chloride or magnesium sulfate, pro-

duced a 19.5-fold decrease in the binding of SN-12868 by the ribonucleate anion. This suggests that the interactions of cationic acridines and quinolines with polyvalent nucleate anions involve, at least in part, the phenomenon treated theoretically by Danielli (*3*). However, the

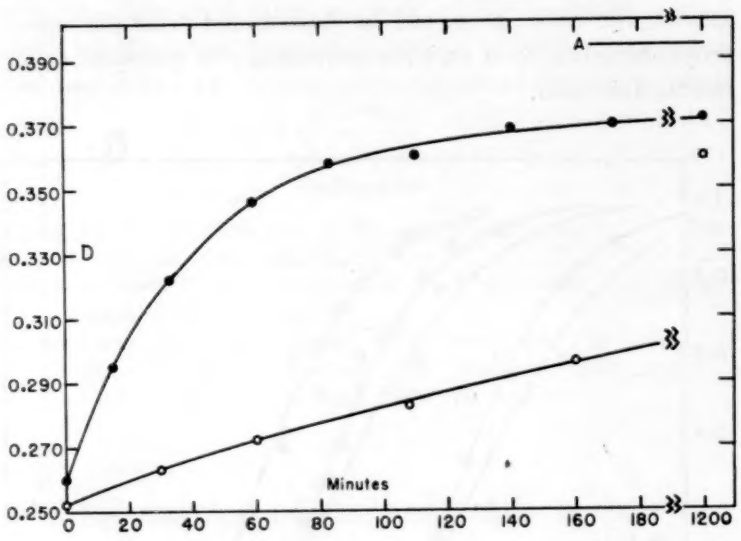

FIG. 3. Change in binding of SN-12868 by ribonucleate during incubation of the latter in 0.5 N NaOH at 24° C (●), and in 0.05 N NaOH at 37° C (○).

difference in binding of SN-12868 and SN-7618 suggests that forces such as hydrogen bonding and van der Waals' forces, are involved in addition to coulombic forces. The interactions reported here should be compared with those between nucleic acids and streptomycin which have been described by Cohen (*2*), and von Euler and Heller (*4*).

That the interaction of these antimalarials with nucleic acids is dependent upon the molecular size of the nucleic acids is suggested by experiments summarized in Fig. 3. Ribonucleic acid was incubated in aqueous solutions of sodium hydroxide. At intervals, aliquots of the solution were transferred to phosphate buffer, HCl equivalent to the alkali in the aliquot was added, SN-12868 was introduced, and the mixed solution was diluted with phosphate buffer in such manner that in every case the solution was 4×10^{-5} M with respect to SN-12868 and contained the equivalent of 0.046 g of ribonucleic acid per 100 ml. Final pH was 5.9 in each case. Optical densities were determined at wavelength 425 mμ. The progressive increase in optical density (Fig. 3) corresponds to progressively diminished binding of SN-12868. The rate of change in 0.5 N NaOH at 24° C was much greater than the rate in 0.05 N NaOH at 37° C, but after 20 hr the optical densities of these two solutions appeared to be approaching an equivalent limiting value, which was less than the optical density (0.394) that would have been observed in the complete absence of binding of SN-12868. The bridging phosphate ester linkages of nucleic acids undergo hydrolytic cleavage in alkaline solution, and these experiments suggest that the binding of acridine derivatives can be used as an indirect measure of such cleavage. Application of these observations in the study of the action of various nucleases is suggested.

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A Rationale for Plasma Therapy in Poliomyelitis

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During the 1948 epidemic of poliomyelitis in Los Angeles, a research program was suggested by one of us (R.M.E.), and initiated by both, in which it was observed that the serum albumin level was frequently lower than normal, and that there seemed to be a correlation between the degree of reduction and the severity of the disease. Because of the importance of serum albumin in maintaining the normal osmotic pressure of blood and of the osmotic pressure in regulating the equilibrium of tissue fluids, and because of the possibility that edema of the cord may contribute to the pathogenesis of the paralysis in poliomyelitis, the suggestion was made that a detailed study be conducted to determine: 1) the alterations in serum proteins which occur during the course of the disease, and 2) the effect on both the serum proteins and the neurologic residual of paralysis as a result of intensive administration of plasma. The results obtained were highly suggestive of a beneficial effect of plasma and will be reported in the near future, with the Los Angeles County Hospital group. It was thought desirable to describe briefly the theory that prompted the suggestion, in the hope that other workers will be stimulated to explore these therapeutic possibilities.

Because the spinal cord is encased in a bony framework, it is particularly susceptible to injury from the edema which consistently accompanies the virus-induced nerve cell damage. Any decrease in serum albumin caused either by accelerated protein catabolism or disturbed protein synthesis and occurring as one of the systemic manifestations of the disease would lower the plasma osmotic pressure and accentuate the spinal cord edema. Conversely, if the serum albumin (and osmotic pressure) were raised by the administration of plasma, the process might be reversed.

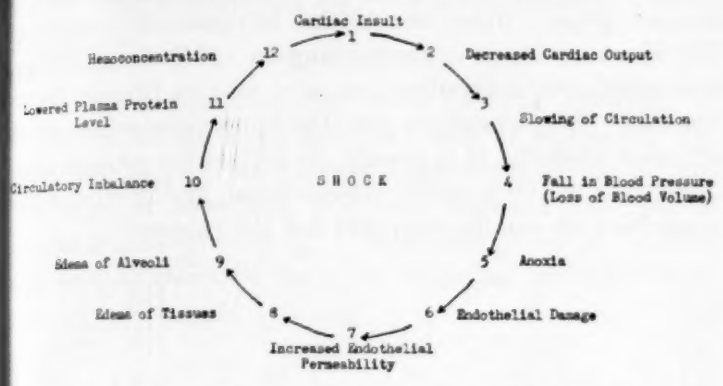

FIG. 1. It is probable that the shock state may be produced by a type of chain reaction initiated at any one of the above 12 points. Hemorrhagic shock emanates from point 4 as an actual loss of blood volume. Infectious shock would start at point 6 with endothelial damage.

In uncomplicated hypoproteinemia, the osmotic pressure of plasma proteins must be reduced to approximately 20 mm of mercury or below before edema is clinically evident. However, when factors tending to precipitate shock are operative, much more moderate decreases in plasma protein may become important in the pathogenesis of generalized or local edema. For instance, it has been shown by Eaton (*2*) that after an acute hemorrhage is induced in dogs, a degree of pulmonary edema rapidly develops which seems to be the result of a shock syndrome, in which there is a decrease in cardiac output, slowing of the circulation, drop in blood pressure, anoxia, endothelial damage, increased permeability of endothelium, transudation of fluid through the impaired endothelial structure, lowering of serum protein levels, and subsequent pulmonary edema (Fig. 1). In these experiments only a slight lowering of the already depressed serum albumin level existent in the shock state was necessary in one group of hemorrhaged dogs to accelerate significantly the accumulation of free fluid in the pulmonary tissue as compared with a control group of hemorrhaged dogs in which no added alteration of serum albumin was made. In poliomyelitis, on the other hand, it is postulated that the virus-induced infection itself may initiate a similar, although at first local, shock syndrome (infectious shock) at point 6 in the diagram, and cause damage to capillary endothelium, vascular congestion, and anoxia, and consequently lead to the transudation of fluid across the impaired endothelium and into the cord. Here again, slight lowering of the plasma osmotic pressure, caused by increased protein catabolism and disturbed protein synthesis as a systemic manifestation of the disease, could lead to a prompt increase in both the degree and the rate at which the local edema of the cord develops.

According to reports in the literature, plasma in moderate amounts has been given in the past to patients with poliomyelitis, with the idea of introducing the immune bodies of the globulin fraction. In one brief study reported by Barnum and Bower in 1944 (*1*), large amounts of plasma were intravenously administered with apparently good results, but again with the apparent intent to administer γ-globulin. The present paper suggests that the therapeutic value of plasma in poliomyelitis may more correctly be attributed to the albumin fraction and to its effect in reducing cord edema. Our preliminary results with a group of 76 Los Angeles patients, soon to be reported, are in accord with this hypothesis and indicate that generous amounts (600 to 900 ml daily) are required.

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A Photoelectric Microdensitometer

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Department of Biology, Washington Square College of Arts and Science, New York University

The photoelectric microdensitometer was designed for the semiquantitative determination of colorimetric histo- and cytochemical tests. Visual judgment of color intensity is not sufficiently reliable to permit comparison of the degree of color in experimental and control preparations. This instrument completely eliminates the subjective element of judging color intensities. It is possible to duplicate the values of different sets of tests (e.g. the Feulgen reaction) on comparable material within ±2%.

The microdensitometer is based on ideas from a number of sources, particularly Dempsey *et al.* (*2*), and Stowell (*3*). Although this densitometer assembly is not new in principle, its adaptability and reliability make it a valuable laboratory aid.

As shown in Fig. 1, the assembly consists of a monocular microscope, a Leitz Makam with a ground glass back, and a model 512 Photovolt Electronic Photometer with a Type C search unit. The phototube search unit is mounted in a traversing mechanism which can be used to move the search unit over the ground glass screen. This assembly in turn is mounted on an arm that can be moved up and down a vertical post. The arm is counterbalanced by a spring-activated steel tape fixed to the top of the vertical post and is provided with a collar which will lock the assembly at any height by a simple set screw. Details of the arm and traversing mechanism are shown in Fig. 2.

Illumination of the Kohler type, as described by Copeland (*1*), is provided by any good microscope lamp and

[1] U. S. Public Health Service, National Cancer Institute Research Fellow.

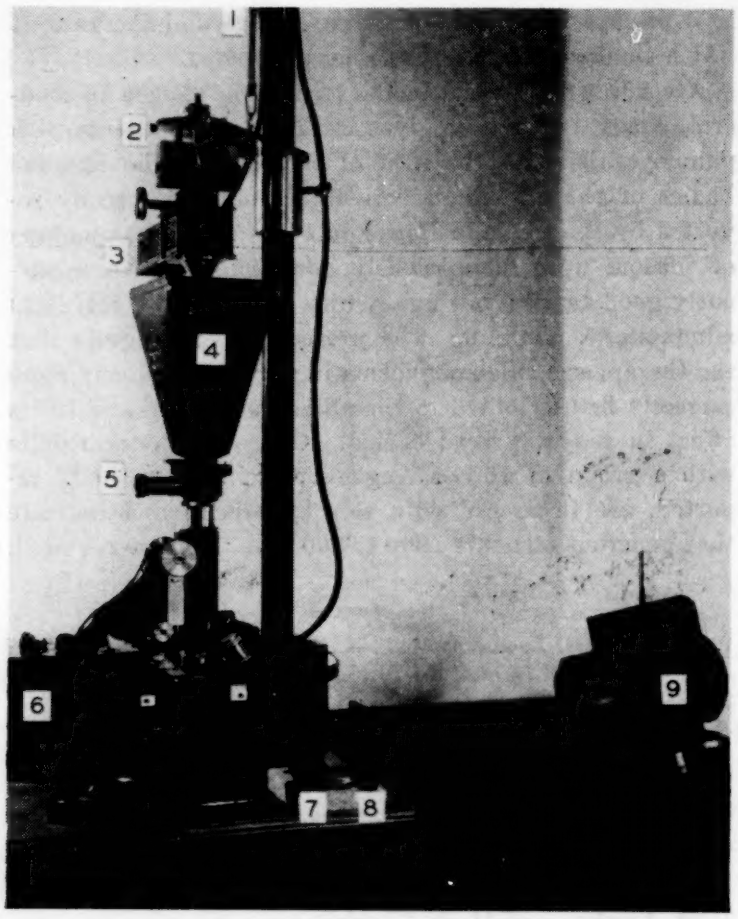

FIG. 1. Photoelectric Microdensitometer. 1. Spring-activated counterbalance. 2. Arm and traversing mechanism. 3. Search unit. 4. Leitz Makam. 5. Side ocular. 6. Photovolt Electronic Photometer. 7. Polaroid variable density screen. 8. Color filter. 9. Lamp housing.

may be adjusted in intensity by a polaroid variable density screen.[2] A 90° prism[2] cemented with Clarite on the flat surface of the microscope mirror converts the light reflecting system, in effect, into a first-surface mirror. This eliminates the secondary light images formed by an ordinary or back-silvered mirror.

The Leitz Makam is used because it can be mounted directly on the microscope, thereby reducing the bulk of the equipment. Furthermore, this camera is equipped with a side ocular for critically centering and focusing the material to be studied. Almost any other type of microscope camera with a ground glass back can be used.

The traversing mechanism for the phototube was made from two rack and pinion units mounted at right angles to one another (see Fig. 2). The search unit was clamped between two brass plates held together by four long screws. The lower plate was provided with an opening slightly larger than the window of the search head, allowing the use of interchangeable aperture cones which adapt the instrument for reading areas of different sizes. These aperture cones used in conjunction with low, medium, and oil immersion objectives make possible densitometric readings of large tissue areas, individual cells, and even small areas within a cell such as the nucleus.

In practice, a filter complementary to the color of the material studied was used. The light intensity was adjusted by means of the polaroid screen until the light passing through a clear area of the slide read approximately 100 on the densitometer. Then the densitometer was adjusted so that it read exactly 100. The section to be studied was then placed in the field, accurately focused, and read by recording the meter readings of specific areas of the section as the search unit (with suitable aperture cone) was placed over parts of the section as seen on the ground glass. Since the initial adjustments constitute the blank readings corresponding to 100% transmission, the subsequent determinations were read as percent transmission. Where controls give the approximate zero point of color intensity, it is possible to adjust the control reading either to 100% or 0% transmission, and then read the experimental results corrected for the controls.

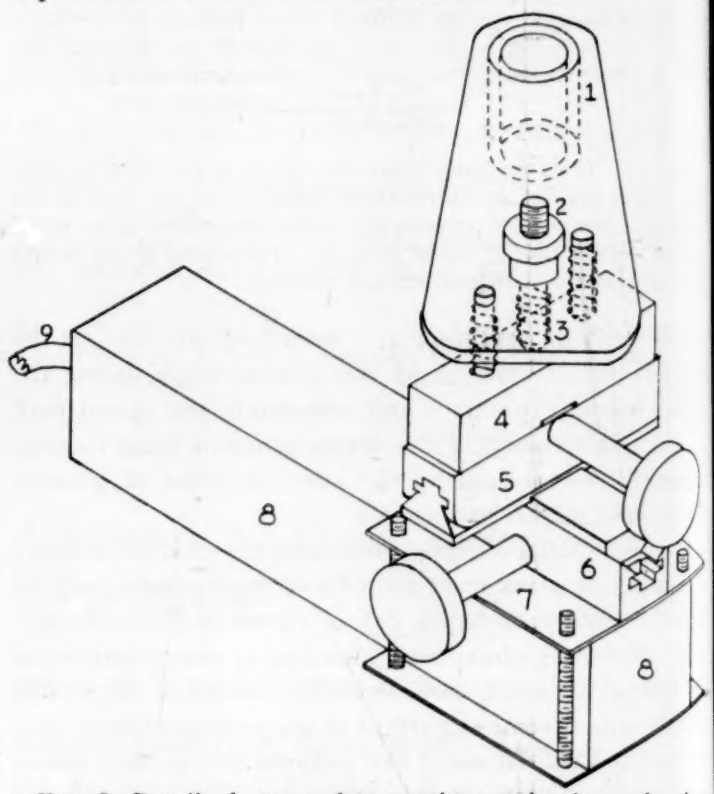

FIG. 2. Detail of arm and traversing mechanism. 1. Arm with collar. 2. Screw mechanism for raising and lowering search unit. 3. Springs. 4. Brass block. 5. Transverse rack and pinion. 6. Longitudinal rack and pinion. 7. Clamp for search unit. 8. Search unit. 9. Cord to electronic photometer.

Readings do not correspond to specific absorption as determined by spectrophotometry where transmission of individual wavelengths can be determined. They can, however, be used as an accurate estimate of the intensity of a reaction provided that experimentals and controls are prepared under exactly comparable conditions. The readings may be used to construct charts and graphs which facilitate the interpretation of results.

The photoelectric microdensitometer can be adapted for use in microchemical determinations. Depression slides may be used as microcuvettes to prepare a set of standards of known concentration for calibration of the instrument. Curves of these values may be plotted and used to determine unknown concentrations as found in densitometer readings of tissue sections, if the difference between the thickness of microcuvettes and of tissue sections is known.

[2] Available from Army surplus material.

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Remote Pipetting Apparatus for Dispensing Solutions of Radioisotopes[1]

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A remote pipetting apparatus for the handling of "hot" solutions is an essential item of equipment for most radioisotope laboratories. Although equipment for this type of operation has been reported (*1*) and is available commercially (*2*), the remote pipetting apparatus herein described is more versatile, provides truly remote operation, and can be assembled from materials more generally available in the laboratory.

The unique feature of this apparatus is the use of a manometer for measuring the delivered volume. This manometer can be placed at the site of the operator at any desired distance from the radioactive source. Filling and delivery from the pipette are accomplished by turning a micrometer screw attached to the plunger of a syringe. The complete apparatus is shown in Fig. 1.

The main parts of the apparatus are the aluminum syringe assembly, the manometer and scale, the pipette support and arm, and the pipette. The syringe assembly consists of a syringe, *A*, (20 ml), held by a strap, *B*, in a semicircular grooved support, *C*; this support can be moved forward or backward on rod *D*, between the arms of the angle block, *E*. The micrometer screw, *F*, is connected to the syringe plunger, *G*, by means of a tubular rubber connection, *H*. The tip of the syringe is connected to a glass Y tube, *I*. One of the forks of the Y tube is connected to the manometer, *J*, the other to stopcock, *K*. This stopcock is used to adjust the level of the colored water in the manometer. One-mm cross-section paper, *L*, is mounted on a board behind the manometer. The other end of the manometer is connected to a straight length of glass tubing, *M*, by rubber tubing, *N*. The glass tubing in turn is connected to a 1-ml pipette, *O*. The supports, *P*, and the horizontal rod, *Q*, can be set at any height or length. The container or receiver, *R*, can be set behind lead bricks (not shown).

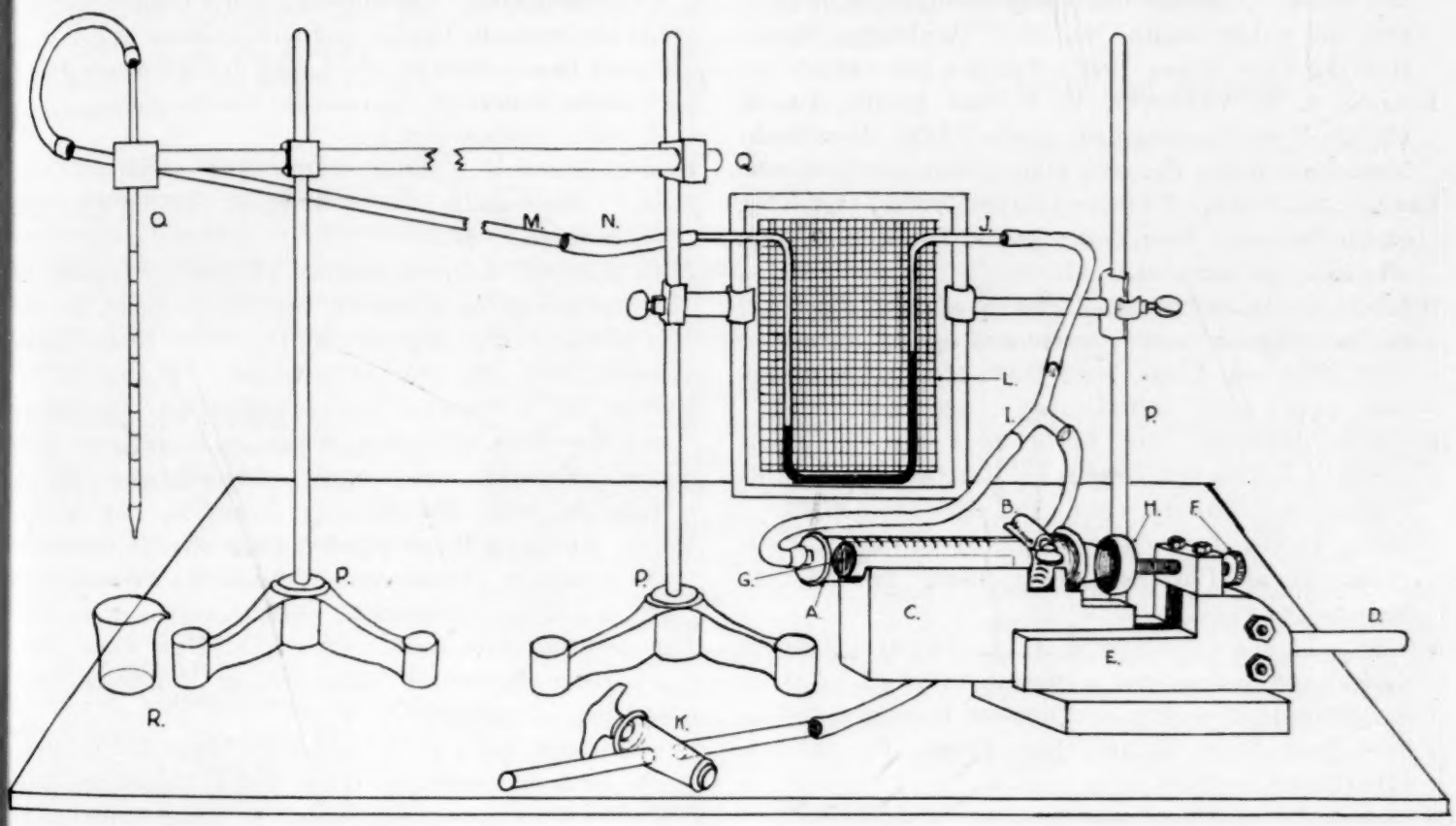

FIG. 1.

Accurate delivery over the entire range of the manometer can be obtained only by using carefully selected tubing of uniform bore for the manometer and by having

TABLE 1

VOLUMES DELIVERED FROM THREE DIFFERENT SECTIONS OF THE MANOMETER SCALE AND THE REPRODUCIBILITY OF REPLICATE SAMPLES*

Section of manometer used	Weight of water delivered	Volume of water delivered at 25° C	Standard deviation σ
	mg/100 mm	ml/100 mm	
215 mm–155 mm	556	0.558	± 0.002 ml
155 mm– 95 mm	563	0.565	± 0.014 ml
95 mm– 35 mm	560	0.562	± 0.007 ml
Mean		0.560	± 0.01 ml

* Four replicates determined for each section.

[1] Published with permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the authors.

all joints leakproof. If uniform bore tubing is not used, then selected sections of the manometer should be used for delivery. The apparatus is calibrated by weighing portions of water delivered by the pipette. Table 1 shows the reproducibility of the volumes delivered, using various sections of a manometer made from random laboratory glass tubing. The accuracy of the delivered volumes compares favorably with that obtained by hand pipetting from calibrated pipettes.

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NEWS and Notes

E. N. da C. Andrade, Quain Professor of Physics at the University of London, will become director of the Royal Institution of Great Britain and resident professor and director of the Davy Faraday Research Laboratory on January 1. Dr. Andrade succeeds **E. K. Rideal,** who has been head of the Royal Institution since 1946.

Ju Chin Chu, director of research in the Department of Chemical Engineering at Washington University, St. Louis, has been appointed associate professor of chemical engineering at Polytechnic Institute of Brooklyn.

Raymond B. Montgomery, of Woods Hole Oceanographic Institute, has been appointed professor of oceanography at Brown University, Providence, Rhode Island. This is the university's first course of instruction in oceanography.

P. B. Medawar, professor of zoology at the University of Birmingham, England, has been appointed John M. Prather Lecturer in Biology at Harvard University this fall. During the first three weeks of November, Dr. Medawar will give a series of six lectures on "Growth, Individuality, and Ageing."

Charles M. Slack has been made technical director of the Westinghouse Electric Corporation's Atomic Power Division, which is now working on an atomic power plant for naval ship propulsion in cooperation with the Argonne National Laboratory of Chicago.

Howard A. Jones has been named assistant director of laboratories for U. S. Industrial Chemicals, Inc., New York City. He will be located at the research and development laboratories in Baltimore.

Victor A. Greulach, botanist in the Department of Plant Physiology, Agricultural and Mechanical College of Texas, has been appointed associate professor of botany at the University of North Carolina, Chapel Hill.

H. Lowell Olson, former University of Wisconsin physicist, has joined the staff of the Applied Physics Laboratory of Johns Hopkins University at Silver Spring, Maryland.

Visitors to U. S.

Christopher Howard Andrewes, head of the Department of Bacteriology and Virus Research, National Institute for Medical Research, Hampstead, London, will lecture at the Harvard Medical School, November 3, 8, and 10. The lectures, to be presented under the Edward K. Dunham Lectureship for the Promotion of the Medical Sciences, will deal with the subject of viruses.

Carl F. von Weizsäcker, professor of physics at the University of Göttingen and at the Max Planck Institute, has arrived here to spend the fall and winter quarters as Alexander White Visiting Professor at the University of Chicago. Dr. von Weizsäcker will present a series of public lectures on "The Modern Concept of Nature" and teach on the staff of the university's Committee on Social Thought.

Margaret Hardy, of Strangeways Laboratory, Cambridge, England, will be a guest research worker in the Tissue Culture Laboratory of the University of Texas Medical Branch, Galveston, in January, 1950. Dr. Hardy plans also to visit the Dermatology Laboratories of Harvard Medical School at the Massachusetts General Hospital, Boston.

Attilio Rampoldi, Italian surgeon and specialist in physical medicine at the University of Rome Clinic, has begun five months' study at the Columbia University College of Physicians and Surgeons. Brought here through efforts initiated by the Foster Parents Plan for War Children, Dr. Rampoldi will study the most recent techniques for treating disabled children. The university contributed a scholarship and the Columbian Civic Club of Newark provided $3,000 toward Dr. Rampoldi's traveling and living expenses.

Gerhart Kessler, visiting instructor from Stuttgart Institute of Technology, Germany, is studying procedures of engineering education at Illinois Institute of Technology this month. Dr. Kessler is also engaged in electrotechnical research at Stuttgart Institute. His visit here is being sponsored by the U. S. Office of Military Government for Germany and the Federal Security Agency's Office of Education.

Grants and Awards

The Farrer Memorial Medal for 1949 has been awarded to W. L. Waterhouse, research professor of agriculture at the University of Sydney, Australia. Dr. Waterhouse received the medal in recognition of his research on wheat diseases and their control.

The Jane Coffin Childs Memorial Fund for Medical Research granted to Gray H. Twombly, Department of Cancer Research, College of Physicians and Surgeons, Columbia University, $5,000 for studies on the selective localization of radioactive estrogens and allied substances in normal and cancer-bearing tissues. It was erroneously stated in the August 19 issue of *Science* that this grant had been made to Frances L. Haven, Biochemistry Department, University of Rochester School of Medicine and Dentistry. Dr. Haven's grant of $3,000 was for studies on the storage and mobilization of fat in tumor-bearing rats.

The Dazian Foundation of New York has awarded a research fellowship to Albert S. Gordon, associate professor of biology at New York University. Dr. Dazian will conduct research on mechanisms underlying the effects of endocrine factors in hemopoiesis.

Theodore Koppanyi, professor of pharmacology and materia medica, Georgetown University School of Medicine, has been elected an honorary member of the Society of Pharmacology and Therapeutics of the Argentine Medical Association.

Fellowships

The Medical Fellowship Board of the National Research Council is now accepting applications for postdoctoral research fellowships for 1950–51 under the following programs:

National Research Fellowships in the Medical Sciences, supported by grants from the Rockefeller Foundation, provide opportunities for experience in research in all branches of medical science. They are open to citizens of the U. S. or Canada who hold the M.D. or Ph.D. degree. As a rule, candidates should be under 30 years of age.

Welch Fellowships in Internal Medicine, also supported by the Rockefeller Foundation, provide a prolonged period of advanced training to persons of proven research ability. They are open to physicians under 40 years of age who are citizens of the U. S.

Fellowships in Poliomyelitis and related fields are supported by the National Foundation for Infantile Paralysis, Inc. They provide training and experience in the study of virus diseases, and in any field contributing to progress in orthopedic surgery. Candidates for fellowships in virus diseases must hold the M.D. or the Ph.D. degree. Fellowships in orthopedic surgery are open only to graduates in medicine who have completed one or more years of hospital work in clincal surgery. Applicants must be citizens of the U. S. and, as a rule, should be under 30 years of age.

Senior Fellowships in Poliomyelitis and related fields are also available under grants from the National Foundation for Infantile Paralysis, Inc. They provide, for persons of proved research ability, opportunity for advanced training and research in the fields of clinical neurology, epidemiology, orthopedic surgery, pediatrics, and virus diseases. Applicants must be U. S. citizens under 40 years of age. Senior fellowships in the field of virus diseases are open to physicians and to holders of the doctorate in the basic sciences; applicants in the four other fields must be graduates in medicine.

Fellowships in Anesthesiology have been made available by a grant from the American Society of Anesthesiologists, Inc. They are designed to relate the disciplines of anatomy, biochemistry, pharmacology, physics, and physiology to problems of clinical anesthesia. Applicants must have the M.S. degree and at least one year of hospital experience.

Fellows will be appointed at a meeting of the Medical Fellowships Board early in March, 1950. Applications for consideration at this meeting must be filed before *December 1.* Appointment may begin at any date determined by the board. For further information address the Secretary of the Medical Fellowship Board, National Research Council, 2101 Constitution Avenue, N.W., Washington 25, D. C.

The Solvay Process Division of Allied Chemical and Dye Corporation has established two graduate fellowships for research on additives to concrete at the Engineering Experiment Station of the University of Kentucky, Lexington. The studies will be directed principally toward increasing the workability of concrete through chemical additives.

Meetings and Elections

A symposium on Tropospheric Wave Propagation, sponsored by the U. S. Navy Electronics Laboratory at San Diego, was held at the NEL laboratory on Point Loma during the week of July 25–29. Organized by Charles R. Burrows, director of the School of Electrical Engineering at Cornell University, Ithaca, New York, the symposium attracted between 50 and 60 specialists in this branch of science, from the U. S., Alaska, and Europe.

Following addresses of welcome by Rawson Bennett, director of NEL, and J. P. Maxfield, chief scientist, representatives of various government facilities conducting radio wave propagation experiments gave talks on the work in progress at their various establishments.

The scientific papers which followed were divided into two main groups—those having to do with atmospheric refraction and those having to do with scattering. An additional session was devoted to hydrometeors and atmospheric absorption. A final session consisted of a lively discussion on topics suggested by participants of the symposium during the earlier sessions.

It was generally agreed that the size of the group was ideal for the informal personal participation which contributes so much to the development of science. An informal dinner at the Admiral Kidd Commissioned Officers Mess, and an all-day inspection trip to the Palomar Astronomical Observatory added to the enjoyment of the participants and contributed to the free discussion of the subject matter of the conference among the visiting scientists.

During sessions on refraction, the data obtained by the NEL on propagation over water in the San Diego area and in propagation over the desert in Arizona were presented and discussed at length, together with the data obtained by the Electrical Engineering Research Laboratory of the University of Texas on the angle of arrival of microwaves both in the Arizona desert and along the Gulf of Mexico. These and other data were interpreted theoretically on the basis of the relation of the vertical gradient of the index of refraction to the laws of radio wave propagation. Methods and difficulties of obtaining the meteorological data from the radio data were also presented.

After the presentation of data on the fluctuations and scattering of radio waves in the atmosphere, collected by members of the Naval Research Laboratory and the NEL, Henry G. Booker and William E. Gordon, of the School of Electrical Engineering at Cornell University, presented their theory of atmospheric scattering. This new theory may explain the deviations of long distance tropospheric propagation from that expected on existing theories. While it is still in the formative stage, many of the consequences of the theory were discussed, and experiments for testing it were described.

C. R. BURROWS

The Theory and Organization of Complicated Automata will be the subject of a series of lectures to be

resented December 12–16 at the niversity of Illinois by John von eumann, of the Institute for Adnced Study. The program follows e series presented at the university st year by Douglas R. Hartree, ritish physicist.

The American Meteorological ociety will hold its 30th anniversary eeting in St. Louis, January 3–6. he first two days will be devoted to symposium on air pollution, under e chairmanship of C. A. Gosline, E. I. du Pont de Nemours and ompany, Wilmington, Delaware.

The Pittsburgh Conference on nalytical Chemistry and Applied pectroscopy, sponsored by the Anaytical Division of the American hemical Society's Pittsburgh Secion and the Spectrographic Society f Pittsburgh, will be held at the illiam Penn Hotel, Pittsburgh, ebruary 15–17, 1950. Papers on ll phases of analytical chemistry nd applied spectroscopy are invited nd abstracts in triplicate must be eceived by *November 1*.

Deaths

Knight Dunlap, 73, professor meritus of psychology at the Uniersity of California at Los Angeles, ied August 14 of pneumonia. Dr. unlap was for many years professor f psychology at Johns Hopkins Uniersity and was chairman of the Diision of Anthropology and Psychology of the National Research Council rom 1927 to 1929.

Earnest Stanley Shepherd, 70, reired research chemist at the Geophysical Laboratory of the Carnegie nstitution of Washington, D. C., ied in Washington on September 9. Dr. Shepherd conducted reearch in the application of physical hemistry to rock magmas and the easurement of the temperature of olten lava.

Sigmund Samuel Greenbaum, 59, rofessor of dermatology and syphilology at the University of Pennsylania, died October 3 at his home in hiladelphia. At the time of his eath, Dr. Greenbaum was also atending dermatologist at Mount Sinai nd Philadelphia General Hospitals.

Leonor Michaelis, 74, physical chemist and retired member of the Rockefeller Institute for Medical Research, died October 9 of a heart attack. His most recent work was the discovery of the radical of vitamin E, the antisterility factor. A native of Berlin, Dr. Michaelis was at one time professor of biochemistry at the Medical School of Nagoya, Japan, and later resident lecturer at Johns Hopkins University.

National Health Program, 1949, a transcript of public hearings conducted last May and June by the Senate Labor and Public Welfare Committee, has been published and copies are obtainable from the committee, U. S. Capitol, Washington 25, D. C. The two-volume, 1,247-page transcript contains the testimony of American Medical Association officers, medical deans, economists, labor spokesmen, and others on compulsory health insurance, voluntary prepayment plans, federal support of medical and dental education, hospital expansion, and other national health issues.

Central Scientific Company announces the opening of a new office at 347 Madison Avenue in New York City to facilitate service in that area on scientific instruments, laboratory supplies, and chemical reagents from unlimited stocks.

At a meeting held at the University of Brussels, Belgium, March 28–30, electrochemists from several Western European countries created a new international organization designated as the **Committee of Electrochemical Thermodynamics and Kinetics.** The goal of the committee is to facilitate and promote international collaboration in these fields. In each country where collaboration is secured, there will be a delegate in charge of coordinating the various activities within the program of the committee.

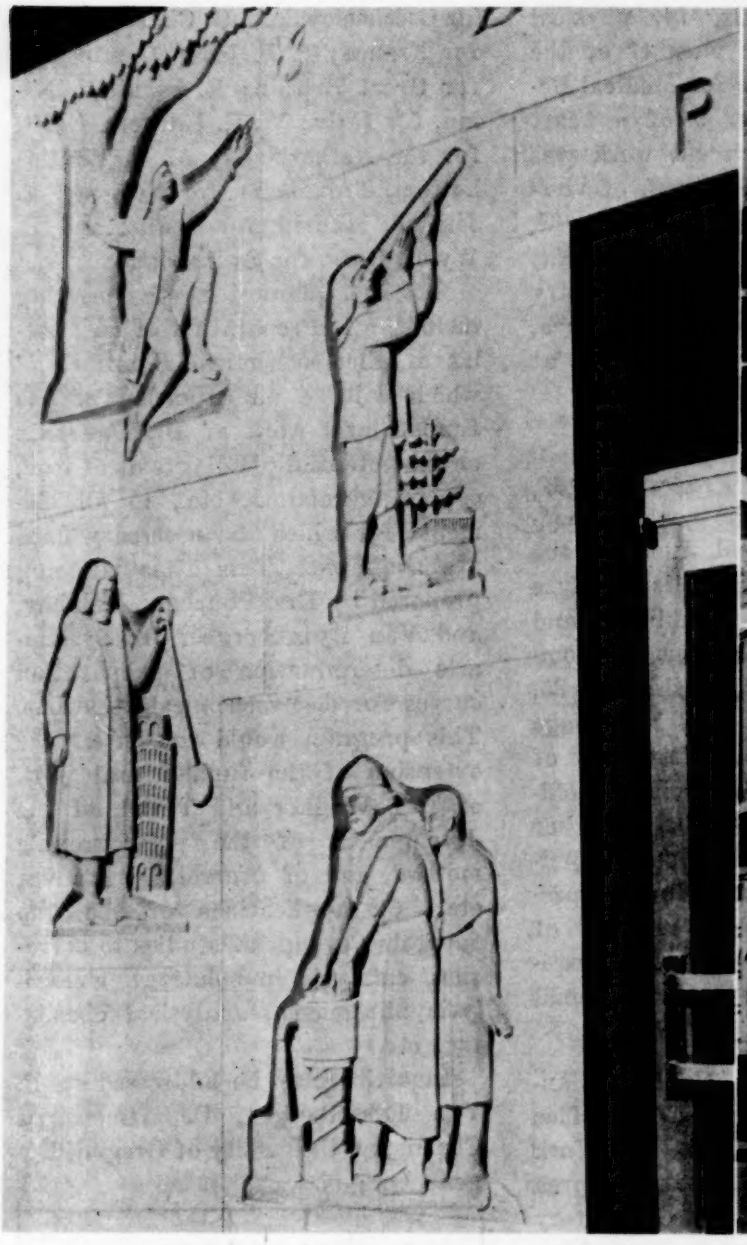
The officers of the committee are: Pierre Van Rysselberghe, University of Oregon, president; M. Pourbaix, University of Brussels, secretary. Delegates have been selected as follows: A. Juliard, Brussels, for Belgium; F. E. W. Wetmore, Toronto, for Canada; J. Heyrovsky, Prague, for Czechoslovakia; G. Charlot, Paris, for France; T. P. Hoar, Cambridge, for Great Britain; R. Piontelli, Milan, for Italy; W. G. Burgers, Delft, for the Netherlands; A. B. Winterbottom, Trondheim, for Norway; E. Jimeno, Madrid, for Spain; and E. Wyler, Bern, for Switzerland.

Research planned at the meeting includes: 1. Preparation of an "Atlas of Electrochemical Equilibria," which will be an extension of the fundamental work of Dr. Pourbaix on the potential-pH diagrams of iron, copper, chromium, etc., to all elements for which the necessary data are available. This atlas is being prepared by Drs. Pourbaix, Delahay, and Van Rysselberghe. 2. Systematic determination of polarization curves for electrochemical reactions. This program would consist of the extension of the fundamental work of Drs. Wagner and Traud, of the development of the polarographic method, and of overvoltage studies, etc. 3. Applications of the two foregoing groups of studies to corrosion, catalysis in solutions, electrolytic phenomena, analytical chemistry, etc.

Inquiries may be addressed to P. Van Rysselberghe, Department of Chemistry, University of Oregon, Eugene, Oregon.

Status of AAAS Meeting Reservations

The early and heavy demand for *single* rooms already indicates a potential shortage of this type of accommodations at the **116th Meeting of the AAAS in New York City,** December 26–31. There are plenty of hotel rooms but many have double or twin beds. Miss Sylvia T. Peltonen, Manager, Housing Bureau, New York Convention and Visitors Bureau, 500 Park Avenue, New York 22, who is in charge of room assignments, urges that, when possible, room reservations be placed by parties of two or more. This also has the advantage of a lower cost per person. Room reservation coupons will appear in the advertising pages of *Science* at frequent intervals.



A unique feature of the new $1,700,000 **Michigan State College Mathematics and Physics Building** is the stone carving on the main and side entrance panels, which tells the history of mathematics and physics. Designed by Carl L. Schmitz, New York sculptor, the work is done in sunken relief, a method widely used by the ancient Egyptians, and comparatively rare today.

Panels at the left of the main entrance depict Newton under a tree, watching an apple fall, Galileo with a pendulum in his hand, Archimedes immersing King Hieron's gold crown in water, and Huygens holding a telescope.

At the right of the entrance Franklin is shown flying his kite, Faraday contemplating a cell through which an electric current is being passed, Newton and Leibnitz discussing a problem by the methods of calculus, and Oersted demonstrating the magnetic effect produced by an electric current.

Above the main entrance are various forms of simple machines—from left to right, the lever, the pulley, gears, the screw, and the inclined plane.

The building houses 135 classrooms and laboratories, one laboratory being two stories high, for experiments using very tall equipment. A central control panel permits any desired voltage and frequency of current to be piped to any laboratory. Equipment to be installed includes an electron microscope, x-ray apparatus for analysis of atomic properties, various radio and radar instruments, and a 10-inch telescope on the building's flat roof.

Thomas H. Osgood is director of MSC's Division of Mathematics and Physical Sciences.

Make Plans for—

Industrial Minerals Division of the American Institute of Mining and Metallurgical Engineers, November 9–12, Tampa Terrace Hotel, Tampa, Florida.

National Symposium on Air Pollution, sponsored the Stanford Research Institute, the California Institute of Technology, and the Universities of California and Southern California, November 10–11, Huntington Hotel, Pasadena, California.

International Symposium on High Altitude Biology, November 23–30, Lima, Peru.

Pittsburgh Conference on X-Ray and Electron Diffraction, November 7–8, Mellon Institute of Industrial Research, Pittsburgh, Pennsylvania.